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Analysis of volatile compounds in a value-added jerky by incorporating Ajwain and Thyme essential oils

MASTER'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled “**Analysis of volatile compounds in a value-added jerky by incorporating Ajwain and Thyme essential oils**” independently, all texts in this thesis are original, and all sources have been quoted and acknowledged by means of complete references and according to the Citation rules of the FTA.

In Prague 25 April 2024

……….……………………..
Elaine Anit

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Abstract

Practical applications of essential oils in food processing and preservation are being investigated by the food industry. Recent interest has been generated in essential oils due to their broad array of significant biological effects on food and human health. The bioactive components found in essential oils are that contributes to their therapeutic and antibacterial properties. Bioactive compounds are inherent in Ajwain essential oil (AEO) and Thyme essential oil (TEO), and these compounds are vital to the development and growth of the plant. Multiple studies have revealed that these compounds provide biological advantages for organisms including humans and animals. Terpenoids and terpenes, which are bioactive compounds present in essential oils, have aromatic and antibacterial properties, rendering them potentially effective natural preservatives in the food industry. The objective of this research was to examine the potential impact of essential oils on the sensory attributes and chemical composition of dried meat. Oil treatment (OT) and hot air blanching (HAB) were performed on the meat samples at concentrations of 0.75 mL and 1.5 mL, correspondingly. Following each treatment, the samples underwent a drying process at a temperature of 55°C for a period of 6 hours. Utilizing gas chromatography-mass spectrometry (GC/MS) and headspace solid-phase microextraction (HS-SPME), volatile chemicals were identified and quantified. The prevailing compounds prior to and following the interventions were thymol, γ-terpinene, p-cymene, and β-pinene. The results indicated that significant differences in the final concentration of the monoterpenes resulted from the application of AEO and TEO regimens. Nevertheless, the results of the sensorial evaluation suggest that the ajwain and thyme OT samples are comparable with regard to their overall rating. In our beef jerky, ajwain essential oil might be the most suitable substitute for thyme.

Keywords: Thyme essential oil; Ajwain essential oil; HS-SPME-GC/MS; dried meat; bioactive compounds

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List of Abbreviations

AEO	Ajwain essential oil
AHAB	Ajwain Hot Air Blanching
ANOVA	Analysis of Variance
AOT	Ajwain Oil Treatment
AU	Area units
CAR	Carboxen
DVB	Divinylbenzene
EO	Essential oil
FAO	Food and Agriculture Organisation
GC	Gas Chromatography
GPA	General Procrustes Analysis
HAB	Hot Air Blanching
HS	Headspace
ISO	International Organisation for Standardisation
MS	Mass Spectrometry
NIST	National Institute of Standards & Technology
OECD	Organisation for Economic Co-operation and Development
OT	Oil Treatment
PDMS	Polydimethylsiloxane
PTFE	Polytetrafluoroethylene
RI	Retention index
SPME	Solid-Phase Microextraction
TEO	Thyme essential oil
THAB	Thyme Hot Air Blanching
TOT	Thyme Oil Treatment

1. Introduction

Essential oils have recently been given recognition for their multiple significant biological effects on food and human health (Burt 2004; Giarratana et al. 2016; Silva et al. 2021). These contain bioactive compounds responsible for the therapeutic and antimicrobial properties of essential oils. Moreover, the food industry is exploring the practical applications of essential oils in food processing and preservation.

In public discourse concerning sustainable food systems, climate change, and optimal nutrition, meat consumption is among the most controversial and contentious topics (Parlasca & Qaim 2022). The contribution of livestock to enhancing human well-being is a subject of debate, as differing perspectives often result in contrasting viewpoints. There is a growing apprehension regarding the health and environmental consequences of the excessive consumption of livestock products in affluent nations. A rise in spending in middle-income countries exacerbates these issues (OECD & FAO 2023). However, livestock plays a crucial role in sustaining the livelihoods of numerous individuals, especially in nations with low-income levels (FAO 1996). Poor livestock keepers acquire various benefits from livestock, including their crucial role in supporting agricultural production in mixed systems, providing nutrients and revenue, and performing cultural functions. Furthermore, livestock can resist economic and climate disturbances (Salmon et al. 2020). Livestock provide food, money, manure, traction, transportation, financial assistance, and social standing enhancement, among other benefits. The multiple advantages of maintaining livestock demonstrate that animals form a vital and indispensable aspect of social life and nourishment in underprivileged communities (Banda & Tanganyika 2021). Given the importance of livestock, it is important to note its vital role as it can provide sustenance for low-income families to meet their physical and nutritional needs. Although it is not implied that livestock should be the sole source of nutrients, integrating various sources of nutrients and a well-balanced diet is still recommended. Some areas have limited access to electricity supply, and budget constraints challenge most low-income families in rural areas.

Moreover, since ancient times, the preservation of food supplies has been an essential concern for civilization, and it is probably even more important in today's world. Consumers, producers, and government agencies have all placed significant emphasis on ensuring the sustainability and safety of food. Food processing and preservation techniques offer the opportunity to extend shelf-life and food availability for consumption. In relation to this, food additives are widely used to increase or maintain nutritional quality, increase storage quality, reduce waste, retain food quality features, and make food more appealing. Food additives are substances or combinations of substances added to foods during production, processing, packing, or storage (Griffiths & Borzelleca 2005).

An elaborate definition of food additives from the CODEX Alimentarius Commission-Procedural Manual (Nutrition Division 2005) states that *"Any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result (directly or indirectly), in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include contaminants or substances added to food for maintaining or improving nutritional qualities".* Examples are traditional synthetic additives like sodium nitrite and sodium chloride, which increase the shelf life of non-dry or dried meat products (Kim et al. 2021). However, these preservation salts pose health risks if consumed in the long term. Researchers are now exploring novel technologies geared toward sustainability, natural or organic, and nutrient-dense (Aksoy et al. 2019; Kim et al. 2021). Essential oils are one of the products incorporated to extend shelf life, are antibacterial, and provide antioxidants to food products (Faustino et al. 2019). The advancement of food additives derived from natural chemicals that include antioxidant and antibacterial properties is an essential milestone in creating food products that promote health. Organic food additives provide more health advantages than synthetic substances. (Szymandera-Buszka et al. 2020).

In view of this, the study aims to determine and measure the volatile compounds of Thyme essential oil (TEO) and Ajwain essential oil (AEO) within dried meat.

2. Literature Review

2.1. Historical background of Jerky or Dried Meat

Prehistoric individuals encountered a dilemma wherein food supplies were unpredictable, but whenever there was an unexpected surplus, it often went to waste before it could all be eaten. For instance, the Ibaloi, an indigenous people from the northern Cordillera region in the Philippines, smoked the excess meat from their rituals called *Kinuday*. *Kinuday* is an Ibaloi word for smoked meat delicacy (Garambas et al. 2022). It is popular in the highlands because there is often leftover meat from the abundant preparations during Cañao celebrations – a socio-religious ritual of the indigenous people from Northern Luzon in the Philippines where animals are butchered as a sacrifice and feasted on. Freezers and refrigerators do not commonly exist in the area; hence, preserved meat by smoking and drying is kept to make it last longer.

Similarly, there is another smoked meat delicacy that is even more popular today as a protein snack, which is called Jerky. A timeless epicurean masterpiece that has prevailed over the years from its humble beginnings to achieve worldwide recognition and adoration as a snack. Jerky, originating from the Quechua term "*ch'arki*," which translates to "dried, salted meat," has a significant historical background closely tied to survival, exploration, and cultural legacy. It has been developed through individual discovery and enhancement of meat preservation techniques involving dehydration and salting. This snack is made from freeze-dried meat/camel meat and is historically known to occur in the Andean's heat and freezing nights (MacDonald 2019). Jerky played a crucial role in ancient civilisations, such as the Inca Empire and indigenous North American tribes, by providing essential nourishment during extended travels, severe winters, and periods of limited resources (Miller & Burger 2000). With its lightweight and nutritionally dense qualities, jerky became a crucial source of sustenance for nomadic groups, hunters, and explorers. In addition, due to the spread of trade routes and the merging of cultures, jerky consumption became common across different continents, adapting to local tastes and cooking traditions. Various preparation methods have evolved, incorporating a variety of spices, herbs, and techniques to enhance the flavour and shelf life of this convenient treat. The history of jerky reflects its enduring relevance as a culinary emblem, reigniting interest in discovering novel ways to improve its sensory attributes. Within this perspective, the inclusion of essential oils emerges as a modern method, with the possibility of preserving tradition while reinventing the primary essence of jerky for current consumer preferences. Moreover, the practicality of the technique assists in providing more options brought about by the sporadic food supply arising from the global climate crisis.

2.2. Essential Oils

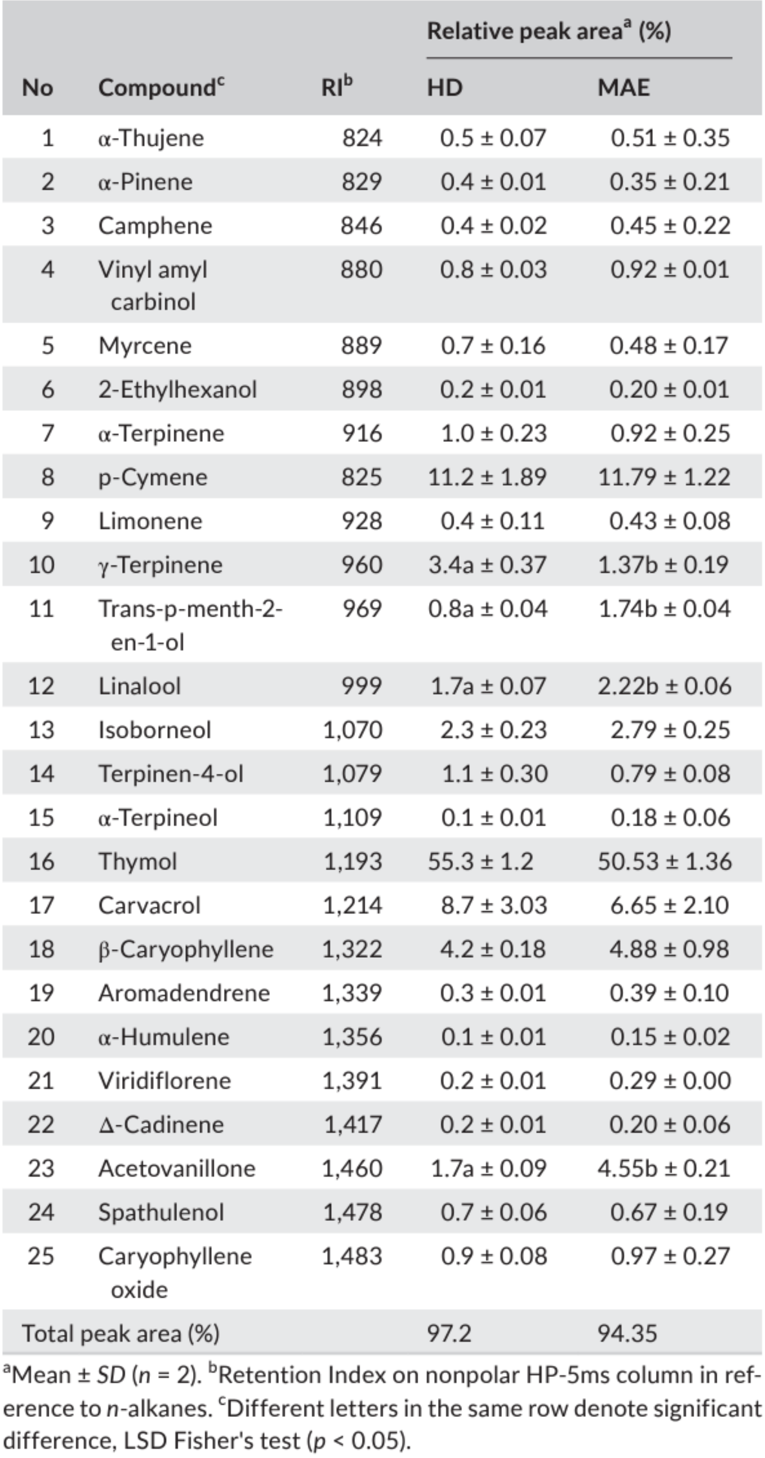
Essential oils have gained recognition for contributing medicinal and health benefits to human well-being. Researchers explored different potential applications of essential oils to food products for the purpose of maintaining or improving the nutritional properties present in the food—nanoencapsulation, edible film, etc. These fragrant, evaporative liquids can be extracted from various plant parts, including flowers, roots, bark, leaves, seeds, peel, fruits, wood, and whole plants (Preedy 2016). Typically, essential oils constitute a small portion of the dried matter of plants (less than 5 %). They are found within specialised secretory structures, including glandular trichomes, oil cells, and secretory ducts or cavities. Extracting volatile oils from various plant parts is possible, including fruits (*Citrus sinensis L.* & *Spondias mombin L.)*, seeds (*Foeniculum vulgare*), grasses (*Cymbopogon citratus),* leaves (*Mentha × piperita L.*), and barks (*Cinnamomum cassia L.)*, flowers (*Rosa abietina*), roots (*Vetiveria zizanoides L.),* woods (*Juniperus virginiana L.*), gums (*Boswellia ameero*), and bulbs (*Allium sativum L.*) (de Sousa et al. 2023).

The variability in the chemical content and quality of essential oils can be attributed to several aspects, including the diversity and nature of molecules, the stereochemistry of constituents, and the extraction methods applied (such as hydrodistillation, effleurages, Soxhlet extraction, cold pressing, and supercritical fluid extraction). In addition, several environmental factors such as climate, cultivation methods, plant organs, harvesting time, light intensity, soil composition, age, and stage of vegetative cycle extraction can also affect the quality, quantity, and composition of extraction products.

Moreover, the application of essential oils varies depending on their purpose and characteristics. Some essential oils are meant for aromatherapy and perfumery, while others are meant for preservation, additional flavour, and aroma in cooking (Aziz et al. 2018). Meanwhile, health concerns regarding the nutritional value, use of synthetic preservatives, and quality production of food are rising, especially since the COVID-19 crisis. People are paying more attention to functional food ingredients and fortified foods to enhance the immune system and combat malnutrition. This aspect also includes recognising bioactive compounds as major contributors to herbal plants' antimicrobial and antioxidant properties (Hassoun et al. 2022). On this account, Masyita et al. (2022) study shows essential oils contain terpenes and terpenoids, which act as the principal bioactive compounds responsible for antimicrobial, antioxidant, anticancer, and anti-inflammatory reactions (Masyita et al. 2022). Given its culinary purpose and innate health benefits, it regained acknowledgement from food manufacturers as an alternative source of natural food preservatives.

2.2.1. Thyme

Thyme, also known as *Thymus vulgaris*, is a perennial herb in the Lamiaceae family. It originated in Southern Europe and is usually grown in an arid temperature and unshaded soil, which is coarse, rough, and well-drained (Patil et al. 2021). This type of soil is often inappropriate for many plant species. The plant has the appearance of short and bushy, and it has several little blooms (Hammoudi Halat et al. 2022). It has a rich history of use in the culinary and medical fields. Thyme, well-known for the fragrant leaves it bears and the robust flavour it imparts, has been an essential component of ancient culinary and medicinal traditions. Essential oil is a concentrated extract from the herb thyme's leaves and flowering tops. It is the fundamental component that gives thyme its distinctive characteristics. For the purpose of harvesting its dried leaves, plant extracts, plant oils, and oleoresins, it is typically produced for commercial purposes in several nations (Silva et al. 2021). In addition to its practical applications in flowering and ornamental purposes, the fragrance and cosmetics sectors use *T. vulgaris L.* due to its distinctive aroma. Moreover, with its powerful aromatic properties, *T. vulgaris* is utilized in the food industry as a flavouring additive for commercial purposes. It is used to preserve several food products such as beef, fowl, and fish. Its significance in the food and pharmacological industries is due to its content of phenol monoterpene derivatives, namely cymene, thymol, and carvacrol as shown in Figure 1 (Silva et al. 2021).



			Relative peak area[a] (%)	
No	Compound[c]	RI[b]	HD	MAE
1	α-Thujene	824	0.5 ± 0.07	0.51 ± 0.35
2	α-Pinene	829	0.4 ± 0.01	0.35 ± 0.21
3	Camphene	846	0.4 ± 0.02	0.45 ± 0.22
4	Vinyl amyl carbinol	880	0.8 ± 0.03	0.92 ± 0.01
5	Myrcene	889	0.7 ± 0.16	0.48 ± 0.17
6	2-Ethylhexanol	898	0.2 ± 0.01	0.20 ± 0.01
7	α-Terpinene	916	1.0 ± 0.23	0.92 ± 0.25
8	p-Cymene	825	11.2 ± 1.89	11.79 ± 1.22
9	Limonene	928	0.4 ± 0.11	0.43 ± 0.08
10	γ-Terpinene	960	3.4a ± 0.37	1.37b ± 0.19
11	Trans-p-menth-2-en-1-ol	969	0.8a ± 0.04	1.74b ± 0.04
12	Linalool	999	1.7a ± 0.07	2.22b ± 0.06
13	Isoborneol	1,070	2.3 ± 0.23	2.79 ± 0.25
14	Terpinen-4-ol	1,079	1.1 ± 0.30	0.79 ± 0.08
15	α-Terpineol	1,109	0.1 ± 0.01	0.18 ± 0.06
16	Thymol	1,193	55.3 ± 1.2	50.53 ± 1.36
17	Carvacrol	1,214	8.7 ± 3.03	6.65 ± 2.10
18	β-Caryophyllene	1,322	4.2 ± 0.18	4.88 ± 0.98
19	Aromadendrene	1,339	0.3 ± 0.01	0.39 ± 0.10
20	α-Humulene	1,356	0.1 ± 0.01	0.15 ± 0.02
21	Viridiflorene	1,391	0.2 ± 0.01	0.29 ± 0.00
22	Δ-Cadinene	1,417	0.2 ± 0.01	0.20 ± 0.06
23	Acetovanillone	1,460	1.7a ± 0.09	4.55b ± 0.21
24	Spathulenol	1,478	0.7 ± 0.06	0.67 ± 0.19
25	Caryophyllene oxide	1,483	0.9 ± 0.08	0.97 ± 0.27
Total peak area (%)			97.2	94.35

[a] Mean ± *SD* ($n = 2$). [b] Retention Index on nonpolar HP-5ms column in reference to *n*-alkanes. [c] Different letters in the same row denote significant difference, LSD Fisher's test ($p < 0.05$).

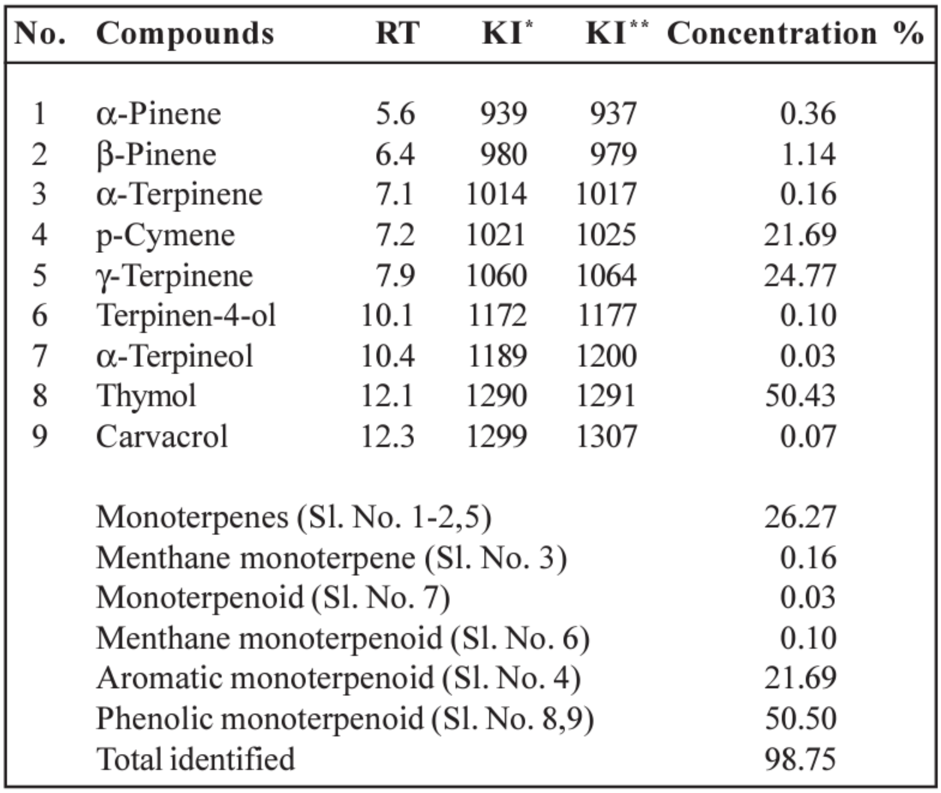
Figure 1. Chemical composition of Thyme (*Thymus vulgaris*)

(Source: Gedikoğlu et al. 2019)

2.2.2. Ajwain

Trachyspermum ammi L., often known as Ajwain, is an annual herb in the family Apiaceae, specifically the genus *Trachyspermum*. It is native to Egypt and is cultivated in several regions: Eastern India, Iran, Pakistan, Iraq, and Afghanistan. Raw Ajwain seeds have a hot and spicy flavor (Bairwa et al. 2012; Dutta et al. 2021). The smell of these plants is similar to that of thyme since thymol is a significant component of their composition (Aziz et al. 2018). Not only has Ajwain essential oil been highly regarded for its potent therapeutic effects, but it has also emerged as a highly sought-after flavouring agent in the realm of culinary operations. The powerful spicy flavour of this ingredient lends a sense of depth to a wide range of meals, ranging from traditional Indian cuisine to contemporary creations from around the world. Over the course of several centuries, Ajwain has made its way into a wide variety of cultural cuisines, where it imparts a flavour profile that is specific to foods while also contributing to the possibility of health benefits. The historical application of Ajwain can be traced back to the old Ayurvedic traditions, after which it gained a reputation for its therapeutic benefits. Due to its digestive, anti-inflammatory, and antibacterial properties, the seeds, especially essential oil, were highly valuable (Grover 2021). The potent chemical composition of Ajwain is responsible for its unique scent and medicinal benefits.

Recent scientific research has provided insights into the possible medicinal uses of Ajwain essential oil. Research indicates that it effectively relieves digestive problems, fights against microbial infections, and demonstrates anti-inflammatory properties (Panda et al. 2020; Dutta et al. 2021; Grover 2021). This has sparked interest in its use in cooking and its potential contribution to health and well-being. One of the main ingredients of Ajwain essential oil, thymol, has remarkable antioxidant and antibacterial capabilities. In addition to p-cymene, terpinene, and limonene, other components enhance the oil's fragrant and medicinal properties as can be seen in Figure 2 (Dutta et al. 2021).



No.	Compounds	RT	KI*	KI**	Concentration %
1	α-Pinene	5.6	939	937	0.36
2	β-Pinene	6.4	980	979	1.14
3	α-Terpinene	7.1	1014	1017	0.16
4	p-Cymene	7.2	1021	1025	21.69
5	γ-Terpinene	7.9	1060	1064	24.77
6	Terpinen-4-ol	10.1	1172	1177	0.10
7	α-Terpineol	10.4	1189	1200	0.03
8	Thymol	12.1	1290	1291	50.43
9	Carvacrol	12.3	1299	1307	0.07
	Monoterpenes (Sl. No. 1-2,5)				26.27
	Menthane monoterpene (Sl. No. 3)				0.16
	Monoterpenoid (Sl. No. 7)				0.03
	Menthane monoterpenoid (Sl. No. 6)				0.10
	Aromatic monoterpenoid (Sl. No. 4)				21.69
	Phenolic monoterpenoid (Sl. No. 8,9)				50.50
	Total identified				98.75

KI*= Kovats Index literature [42]
KI**= Kovats Index experimental

Figure 2. Chemical analysis of essential oil of Ajwain *(Trachyspermum ammi L.)* seed

Source: (Dutta et al. 2021)

2.3. Bioactive Compounds and their Nutritional Benefits

Bioactive compounds also referred to as phytochemicals, are naturally occurring molecules found in plants and other organisms that have specific biological effects on living organisms. According to Frank et al. (2020), they are substances that exhibit effects on biological systems, such as animals and people. Moreover, phytochemicals are substances essential for the functioning and survival of plants. They perform several functions, such as defence mechanisms against potential threats, attracting pollinators, and regulating growth and development (Martinez et al. 2017). Bioactive compounds, on the other hand, are a type of phytochemicals that often impact living organisms including human health—examples are terpenoids, alkaloids, nitrogen-containing substances, organosulfurates, and phenols (Višnjevec et al. 2024). These secondary metabolites that can be found in essential oils possess certain characteristics that enable them to interact with biological systems and produce a physiological response as demonstrated by numerous in vitro and in vivo studies.

Due to these therapeutic properties, there has been a growing interest in bioactive compounds in recent decades due to their potential to enhance health and their utility in the food industry. Bioactive substances influence the nutritional quality, sensory qualities, and functional properties of food products. They affect the colour, flavour, and scent of foods, influencing consumer preferences and acceptability. Furthermore, bioactive chemicals have a variety of physiological benefits, that have received interest due to their possible significance in illness prevention and enhancing overall health and well-being.

In essential oils, four groups of chemical constituents are found namely terpenes/terpenoids, phenylpropanoids, and other constituents (phenols & alcoholic compounds) as shown in Figure 3.

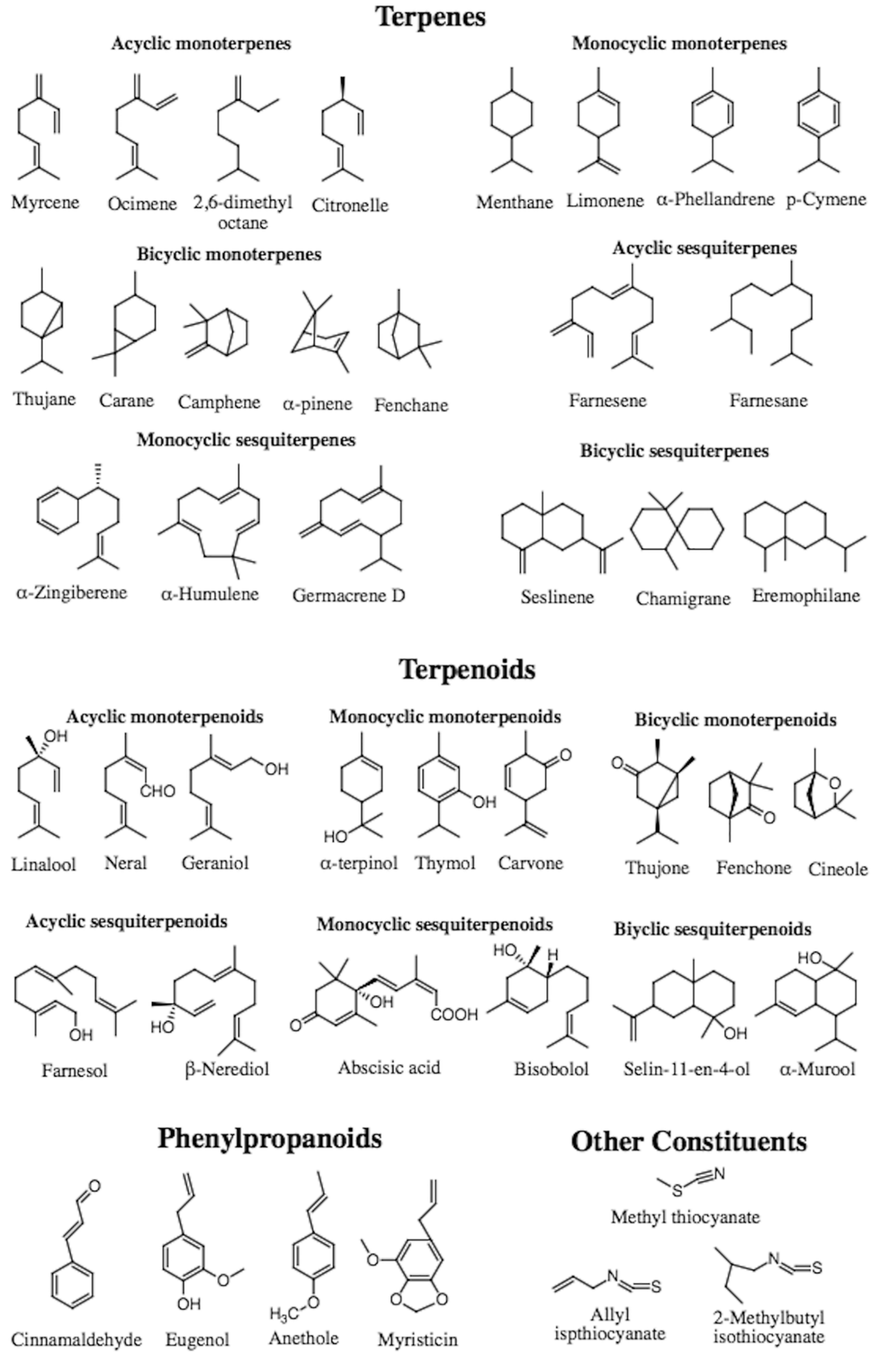


Figure 3. Four groups of chemical constituents found in essential oils
(Source: Masyita 2022)

2.3.1. Terpenes

Terpenes, also known as isoprenoids, are the primary components found in essential oils. They have molecular structures that consist of carbon backbones made up of 2-methylbuta-1,3-diene units (known as isoprene units), which can be rearranged to form cyclic structures. The structural diversity of terpenes is mostly determined by the quantity of isoprene units. Hemiterpenes consist of a single isoprene unit (C5), while monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30), and tetraterpenes (C40) are created by multiple isoprene units (Noriega 2021). Hemiterpenes constitute a small proportion of the terpenes present in essential oils (EOs). Isoprene, the most prominent hydrocarbon terpene (HT), is released into the atmosphere by several trees, including conifers, oaks, poplars, and willows. Hemiterpenes such as angelic, tiglic, isovaleric, and senecioic acids are some examples. Monoterpenes make up the majority of essential oils (90 %), with sesquiterpenes being the next most abundant. Small quantities of diterpenes, triterpenes, and tetraterpenes, together with their oxygenated derivatives, are also present.

2.3.2. Terpenoids

Terpenoids, often known as terpenes, are a broad class of organic substances found in plants, fungi, and some animals. They are formed from the fundamental building blocks of isoprene units and exhibit substantial structural variety, functional adaptability, and biological activity. Terpenoids play important roles in various biological activities, including defense mechanisms, communication, and chemical signaling within organisms and ecosystems. In a recent study by Álvarez-Martínez et al. (2021), terpenes exhibited antibacterial properties against both antibiotic-susceptible and antibiotic-resistant bacteria, mostly by rupturing cells and suppressing protein and DNA synthesis. Masyita et al. (2022) also mentioned the bactericidal effect of carvacrol, eugenol, geraniol, carvone, and thymol against *Staphylococcus aureus.*

In addition, it consists of multiple isoprene units (C_5H_8) are added to oxygen molecules. These compounds are primarily hydrocarbons and are classified into different groups based on their number of isoprene units (Masyita et al. 2022).

Monoterpenoids, for example, are composed of two isoprene units, each comprising 10 carbon atoms and 16 hydrogen atoms ($C_{10}H_{16}$). On the other hand, Sesquiterpenoids consist of three isoprene units, each with 15 carbon atoms and 24 hydrogen atoms ($C_{15}H_{24}$). Diterpenoids, with four isoprene units, have a chemical formula of $C_{20}H_{32}$, while triterpenoids, made up of six isoprene units, are represented by the formula $C_{30}H_{48}$. Each category of terpenoids possesses unique chemical structures and properties, leading to a diverse range of biological activities and applications. As evident in recent studies, these compounds demonstrate antimicrobial activity (Guimarães et al. 2019), antiviral activity, and antifungal activity (de Sousa et al. 2023). Also, therapeutic properties include anticancer, anti-inflammatory, antioxidant, antiallergic, neuroprotective, antiaggregator, anti-coagulation, sedative, and analgesic effects (Masyita et al. 2022).

2.3.3. Phenylpropanoids

Phenylpropanoids are another essential oil constituent biogenetically derived from the aromatic amino acid L-phenylalanine via the shikimate pathway. The shikimate pathway serves as the primary connection between the breakdown of sugar and secondary metabolism. It begins with the reaction between phosphoenolpyruvate and erythrose 4-phosphate, which are products of glycolysis and the phosphopentose pathway, respectively (Wu et al. 2022). It is also the source of aromatic compounds such as aldehyde, phenol, alcohol, methoxy, and methylenedioxy compounds.

In addition, phenylpropanoids are composed of a phenyl ring linked to a C3 propane moiety at their nucleus (de Sousa et al. 2023). Although present in the plant kingdom, their prevalence is comparatively lower than that of terpenes. The remarkable variety of phenylpropanoids is the result of effective modifications to an extremely restricted set of fundamental structures.

2.4. Compositional Analysis of Volatile Compounds

The process of compositional analysis of volatile compounds begins with their isolation. Headspace-Solid Phase Microextraction (HS-SPME) is the commonly used technique for collecting the volatile compounds present in the sample. Once the volatile compounds are collected, they are detected through a separation method called Gas Chromatography (GC). After the compounds are separately detected, Mass Spectrometry (MS) is done to present all the volatile compounds that exist in the sample. The processes are further detailed below.

2.4.1. Headspace-Solid Phase Microextraction (HS-SPME)

Headspace Solid-Phase Microextraction (HS-SPME) is an effective method in analytical chemistry that is employed to extract volatile and semi-volatile chemicals from different sample matrices prior to analysis (Bojko et al. 2011; Roasa et al. 2019). It is widely used in areas such as environmental analysis, culinary science, and forensic chemistry. The procedure commences with sample preparation, wherein the sample is enclosed within a hermetically sealed container and subjected to heat, facilitating the migration of volatile chemicals into the headspace located above the sample. A Solid-Phase Microextraction fiber, selected according to the desired molecules and coated with a specific phase, is subsequently introduced into the headspace to adsorb these volatile compounds (Heaven & Nash 2012). Following an extraction time, the fiber is extracted and promptly placed into the injection port of a Gas Chromatograph (GC) or other analytical instruments for desorption and analysis. Headspace Solid-Phase Microextraction (HS-SPME) provides several benefits, such as its straightforwardness, little manipulation of samples, adaptability to various fiber coatings, heightened sensitivity for detecting small amounts of substances, and cost-effectiveness resulting from less solvent use. HS-SPME is an essential instrument for effectively and sensitively extracting volatile chemicals in complicated samples, playing a vital role in developing analytical chemistry procedures.

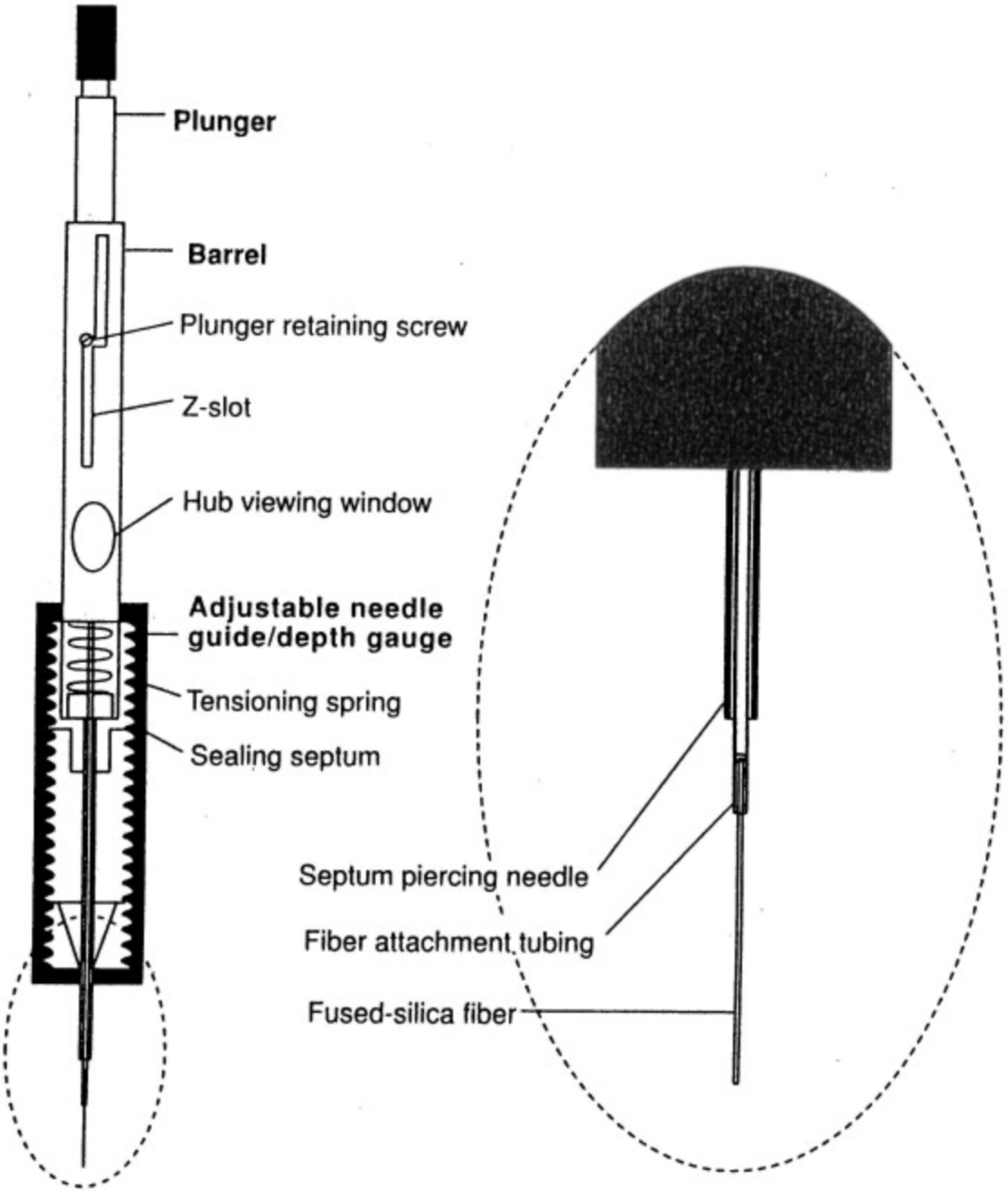


Figure 4. Parts of SPME
(Source: Kataoka et al. 2000)

2.4.2. Gas Chromatography-Mass Spectrometry (GC-MS)

Gas Chromatography (GC) is an analytical technique used to separate gaseous, liquid, semi-liquid, and solid samples (Turner 2022). It also specializes in smaller volatile and semi-volatile organic molecules such as hydrocarbons, alcohols, and aromatics, as well as pesticides, steroids, fatty acids, and hormones, making it widely used in a variety of application areas and industry segments, particularly food safety and environmental testing. When paired with the detection power of Mass Spectrometry (MS), GC-MS instrument (shown in Figure 5) can be used to separate complex mixtures, quantify analytes, identify unexpected peaks, and detect trace levels of contaminants.

Figure 5. Gas Chromatography Apparatus

Source: (Agilent 2024)

In Figure 6, the process involves heating a liquid sample to the point where it undergoes a phase change and transforms into a vapour. This vapour is then transported by a gas such as helium or hydrogen. The carrier gas, also known as the mobile phase, conveys the sample along a lengthy and slender glass or metal tube, referred to as the column, which is covered with a chemical substance known as the stationary phase. As the gaseous chemicals traverse the column, their velocity decreases upon encountering the immobile phase. The duration for different chemicals to reach the end of the column varies depending on their chemical characteristics. Once the compounds are isolated, they are subsequently sent to the Mass Spectrometer (Agilent 2024).

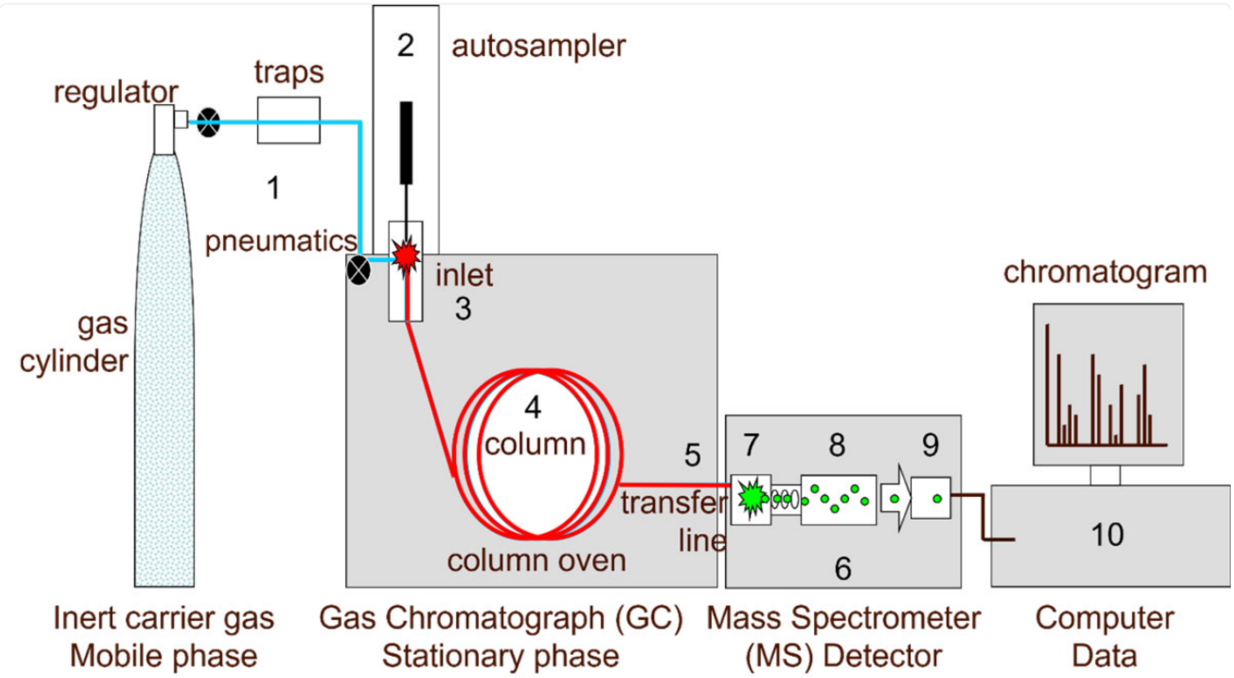


Figure 6. Simplified diagram of HS-SPME GC-MS
(Source: Turner 2022)

2.5. Food Preservation Techniques

Over the course of history, humans have undergone a series of global events brought about by changes in climate, global conflicts, transportation limitations, economic crises, etc. These events have challenged civilisation to face inconsistent and limited availability of sufficient, safe, and nutritious food that fits individuals' dietary needs. Consequently, multiple methods were developed to conserve food, prevent food spoilage, and nourish individuals for a longer period during times of scarcity, long-distance travel, and constraints related to seasonal availability. Food preservation is a critical part of food processing and storage. Its main reasons are preventing immediate food spoilage, prolonging the shelf life, preserving the quality, and guaranteeing the safety of food products. Food spoilage, which refers to a change in the sensory properties of food, can happen at any point in the food supply chain (Onyeaka & Nwabor 2022). The changes in sensory properties of food can be caused by mechanical, physical, chemical, enzymatic, and microbial changes. Microbial changes have the most adverse impact on food and human health because of food spoilage. Undesirable microbial growth and contamination could induce disruptive and detrimental toxins to human health. Thus, the growth rate of these microorganisms in food must be apprehended. The growth rate of undesirable microorganisms is affected by pH, nutrient availability, temperature, moisture content/water activity in food, and oxygen availability. Food items can be preserved through drying, salting, fermentation, freezing, packaging, and many more.

The contemporary food industry utilises a variety of traditional and creative techniques to maintain the quality of food, in response to consumer preferences for ease, freshness, and nutritional content.

2.5.1. Drying

Drying, one of the earliest methods of food preservation, has been utilised to extend the shelf life of meat and other foods for centuries (Brown 2011). Drying meat prevents spoilage by impeding the development of microorganisms through the removal of moisture. There are various meat curing techniques, each with its own benefits and considerations.

Sun drying is one traditional technique of dehydrating meat (Inyang et al. 2017). Thinly sliced or pulverised meat is exposed to the sun's heat and air circulation in a single layer using this technique. The warmth of the sun aids in the evaporation of moisture from the meat, causing a progressive reduction in its water content. However, consistent sunlight and low humidity are necessary for sun drying to occur, which reduces its dependability in specific climates or seasons.

Air drying is another prevalent technique that can be implemented indoors under controlled environmental conditions (Inyang et al. 2017). Salt and spices are commonly used to marinate meat prior to air drying in order to improve flavour and assist in preservation. The meat is subsequently suspended or laid out in an area with adequate ventilation and airflow. The air circulation aids in the removal of moisture from the meat, causing it to dry progressively over time. In contrast to solar drying, air drying offers the advantage of greater control and year-round operation, rendering it a viable method for industrial production (Chellaiah et al. 2020).

The critical factor in achieving effective meat preservation through air drying or sun drying is the reduction of moisture content to a threshold that impedes microbial proliferation. This serves to avert deterioration and prolong the meat's shelf life. In addition to using clean surfaces and equipment, preserving dried meat in airtight containers, and handling meat at safe temperatures, proper hygiene practices are crucial for maintaining food safety.

Dried meat, including jerky and biltong, is a portable and convenient refreshment that maintains a significant portion of its nutritional composition and provides an extended period of storage in contrast to fresh meat. Dried meat can remain viable for months or even years when appropriately dried and preserved, rendering it a highly advantageous method of preservation for both commercial and residential applications (Miller & Burger 2000).

2.5.2. Curing

Curing has been used for centuries as a method of meat preservation to prolong its storage life while simultaneously improving its flavour and texture. Meat is treated with salts, sugars, nitrates or nitrites, and occasionally additional herbs or seasonings, during this procedure (Brown 2011). Curing inhibits enzymatic activity and the development of microorganisms, both of which contribute to deterioration.

Dry curing is one of the primary procedures utilised to cure meat. A solution comprising salt, sugar, and curing agents (e.g., sodium nitrate or sodium nitrite) is directly applied to the meat's surface in this technique. Depending on its size and variety, the meat is then placed in a controlled environment with low humidity and permitted to cure for an extended period of time, typically several weeks to months. The curing agents permeate the meat during this period, extracting moisture and establishing an unfavourable environment for bacterial growth (Chellaiah et al. 2020).

An additional prevalent technique is moist curing, which is also referred to as brining. Wet curing involves submerging the meat in a solution that comprises curing agents, water, salt, sugar, and occasionally seasonings or flavourings. The curing solution aids in the preservation of the meat while also enhancing its flavour and tenderness through absorption. Wet curing is frequently applied to smaller cuts of meat or when a reduced curing time is desired, as it is more rapid than dry curing.

In addition to preserving meat, curing imparts its distinctive flavor and hue. In addition to inhibiting bacterial growth, the addition of curing agents such as nitrates or nitrites helps preserve the pink or reddish hue of the flesh, particularly in products such as cured hams or bacon (Shakil et al. 2022; Amiri et al. 2024).

Following curing, the meat may be subjected to additional procedures, such as smoking or drying, to further enhance flavor and texture. In addition to imparting a discernible smoky taste, smoking offers supplementary preservation advantages by virtue of its antimicrobial characteristics. Moisture is extracted from meat via air drying or controlled drying environments; this process concentrates flavors and increases the meat's shelf life.

The implementation of appropriate curing methods is critical in ensuring food safety. To ensure that harmful bacteria are effectively controlled, it is critical to use the recommended curing durations and temperatures and to apply the correct amount of curing agents. In addition, proper storage conditions must be maintained for cured meat to prevent decomposition and preserve its quality. Cured meats, such as prosciutto, bacon, ham, and salami, are cherished globally due to their opulent tastes and extended longevity; thus, curing remains a traditional preservation technique that has maintained its popularity over time.

3. Aims of the Thesis

The main objective of this study is to explore the potential of using Ajwain and Thyme essential oils as natural flavor enhancers in value-added jerky products. This includes evaluating the chemical composition of the different doses of essential oil in the dried meat, as well as the evaluation of relevant organoleptic properties of the final product.

The specific aims of this paper were to:

a. Identify the volatile compounds of TEO and AEO after meat drying.
b. Quantify the chemical composition of TEO and AEO in dried meat for each blanching methods (Hot-Air Blanching & Oil Treatment) and for various doses (0.75 mL and 1.5 mL).
c. Conduct a sensory analysis to assess the effects of Ajwain and Thyme essential oils on the flavour, aroma, texture, and taste of value-added jerky.

4. Materials and Methods

4.1. Essential oil

Thyme essential oil (TEO) was obtained from Katyani Exports (New Delhi, India). According to the manufacturer’s specifications, TEO was extracted by steam distillation from the entire flowering plant (herb) without the root. Ajwain essential oil (AEO) was purchased from the same supplier and was extracted from the crushed seeds through steam distillation. For the chemical analyses of TEO and AEO, the VOCs were diluted with hexane, and 5 µL TEO was added to 1 mL of hexane. After 10 min of shaking, TEO and AEO were analysed by GC/MS to see all the VOCs contained in the TEO.

4.2. Sample preparation

For this study, fresh beef from biceps femoris was purchased from a local butchery in Prague, Czech Republic. The beef muscle surface was washed and then stored at -6°C for 1 day. After that, the frozen muscle was defrosted at ambient temperature (± 25 °C) for 1 hour and was sliced into thick steaks of 1 cm by a meat cutter SILVER (Kalorik, Miami Gardens, FL, USA). Additionally, it was cut into 5 cm × 2.5 cm small rectangular samples. Meat slices were packaged in zipper plastic bags and stored at - 6 °C in the freezer, as shown in Figure 7.

Figure 7. Slices of meat (5 cm x 2.5 cm) for jerky

4.3. Preparation of modified blanching treatments

Two modified blanching treatments were used to prepare meat samples, using TEO (Thyme essential oil) and AEO (Ajwain essential oil). The doses of these treatments were 0.75 mL and 1.5 mL. Prior to each treatment, the meat sample was immersed in a salt solution containing 12 grams of sodium chloride per liter (12 g NaCl L^{-1}) for 10 minutes. Hot air blanching (HAB) involves saturating a filter paper with varying amounts of essential oil and positioning it in front of the fan within a drier (Memmert drier UFE 400, Germany) with two trays (Figure 9). Subsequently, the meat sample was introduced into the dryer and dried for 10 minutes at a temperature of 35 °C. This resulted in the generation of vapours containing TEO and AEO. Subsequently, for the oil treatment (OT) (shown in Figure 8), the specimen was submerged in 20 mL of sunflower oil together with each essential oil and each prescribed amount at room temperature for 10 minutes. After each blanching treatment, all beef samples were subjected to drying in a Memmert drier UFE 400 from Germany at a temperature of 55 °C for a duration of 6 hours. Upon 3 hours of drying, the samples were rotated to ensure thorough drying of the whole surface.

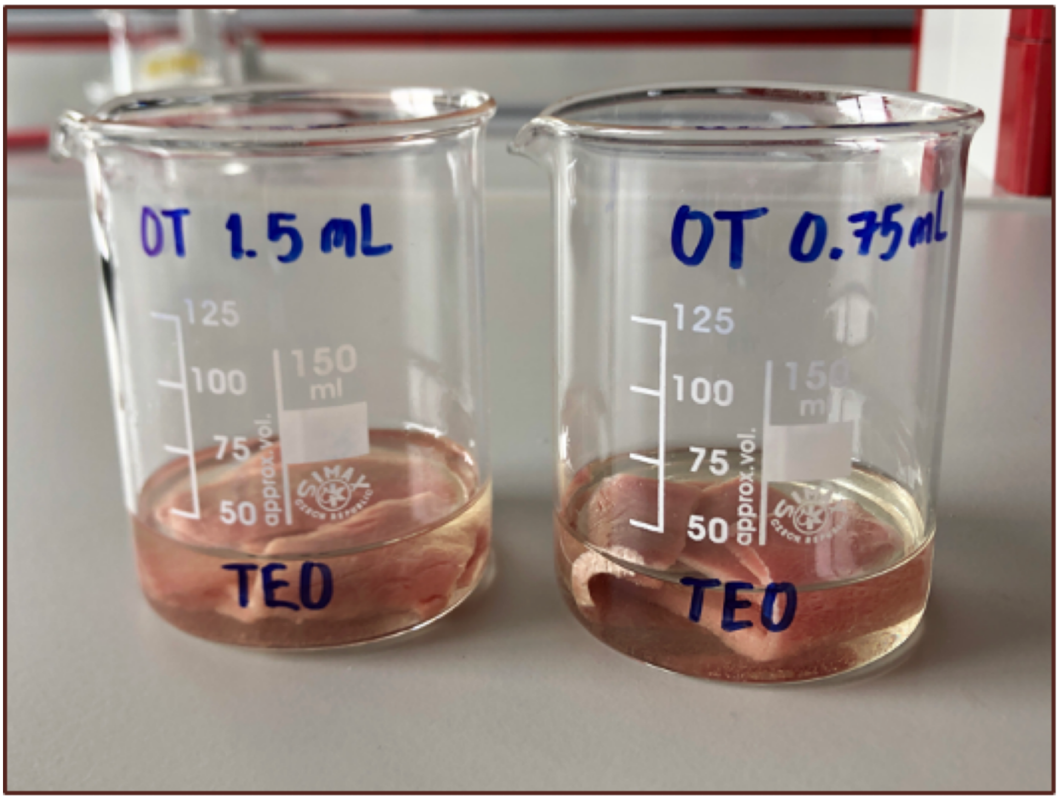


Figure 8. Oil treatment (OT) of samples with Thyme essential oil

Figure 9. Hot air blanching (HAB) of samples

4.4. HS-SPME extraction method

The Solid Phase Microextraction (SPME) technology was employed to extract volatile chemicals from the Headspace (HS). The quantitative relationships between the primary volatile components of TEO and AEO have been identified. Prior to analysis, the fiber was subjected to a conditioning process at a temperature of 250 °C for 60 minutes in the gas chromatograph inlet. After completing the drying process for each modified blanching treatment, a 4 mL vial sealed with PTFE-faced silicone septa was used to weigh 1 ± 0.02 g of ground dried meat. The vial was then injected with an SPME fibre, which had a thickness of 50/30 um and was made of DVB/CAR/PDMS. The fibre was subjected to the gas phase above the sample at room temperature (± 25 °C) for 30 minutes. Following the adsorption process, the fibre was extracted from the vial and promptly placed into the gas chromatography inlet.

4.5. Gas chromatography-mass spectrometry (GC/MS) analysis/quantification

The volatile components of TEO and AEO were quantitatively analysed using gas chromatography-mass spectrometry (GC/MS) on samples subjected to various doses and treatments. The study was performed using an Agilent 7890B GC equipped with an Agilent 5977 MSD mass spectrometer. Following the extraction procedure, the fiber was manually inserted into the injection port of the GC/MS. The temperature was set to 250 °C in spitless mode. The gas chromatography column used was HP/5MS 5 % Phenyl methyl Siloxane with dimensions of 30m length, 250 µm inner diameter, and 0.25 µm film thickness. Helium was utilised as the carrier gas, maintaining a consistent flow rate of 1 mL/min. The temperature of the GC oven was increased in a controlled manner. It started at 45 °C and remained at this temperature for 5 minutes. Then, it was raised to 250 °C at 10 °C per minute. Once it reached 250 °C, it was further increased to 280 °C at a faster rate of 20 °C per minute. The temperature was then held at 280 °C for 5 minutes. The entire analysis process took a total of 32 minutes. Following each injection, the fibre was cleaned at a temperature of 250 °C for 10 minutes to guarantee its impeccable condition. Individual components were identified by comparing their retention times with those of analytical standards and cross-referencing the mass spectral database with the NIST 7 Mass Spectral Library. The relative percentages of the components were calculated based on the gas chromatography peak areas, with only compounds exceeding 0.1 % being considered. Ultimately, three iterations per analysis were conducted, and the outcomes were quantified as area units (AU) multiplied by 106 per gram of dry matter.

4.6. Chemical composition of TEO and AEO (GC/MS)

The chemical content of both essential oils was determined using GC/MS. The essential oil was diluted in hexane at a 5 µm/mL concentration for sample preparation. The sample was injected into the GC/MS Agilent 7890B GC port in split mode, with a split ratio of 10:1. The injection temperature was set to 250 °C, and the carrier gas used was Helium, flowing at a constant rate of 1 mL/min. An identical column to the previous GC/MS quantification method was utilised. The initial oven temperature was set to 45°C and maintained for 5 minutes. It was then increased to 250°C at a rate of 10°C per minute. Finally, the temperature was further increased to 280°C at a rate of 20°C per minute and kept for 5 minutes. The total run duration of the oven was 45.5 minutes. The constituents were identified by comparing their Retention indices (RI) and retention times (RT) with the National Institute of Standards and Technology Library ver. 2.0.f (NIST, USA), as well as authentic standards (Sigma-Aldrich, Prague) and literature (Adams, 2007). The retention index (RI) was determined for compounds separated by the HP-5MS column. The RI values were computed based on the retention durations of a series of n-alkanes ranging from C8 to C40, obtained from Sigma-Aldrich in Prague.

The relative proportions of each constituent were determined by comparing the peak regions with those in the NIST 7 Mass Spectral Library. Compounds with concentrations greater than 0.1 % were included and measured in area units (AU) × 106/g.

4.7. Statistical analysis

The statistical study was conducted using Statistica® Vs.13.5.0.17 software on Windows 10. A total of 60 samples were used in this investigation, with each sample consisting of two dosages, two essential oils, two blanching treatments, and including, two controls per replications. Furthermore, three independent experiments, or replications, were carried out. The normal distribution was assessed using the Kolmogorov-Smirnov and Lillefors tests, while variance homogeneity was verified using the Levene test. The chemical composition (expressed as a percentage of the total area) was analysed using analysis of variance (ANOVA) to determine the major effects of each treatment type. The experimental design included each composition (expressed as a percentage of the total area) as a dependent variable. The categorical parameters in this study were samples, doses, and repetition, all of which were considered simultaneously. The key interaction we utilised in the analysis was the combination of the sample and dosage variables. A Tukey correction was performed for doing multiple mean comparisons. Statistical significance is defined as $p < 0.05$.

In addition, we conducted ANOVA main effects and Tukey's HSD post hoc test to analyse the sensory data and identify significant differences in between the means. Within the model, each assessed characteristic served as the dependent variable, while the categorical factor represented the type of sample. Following an initial assessment using ANOVA, a Generalised Procrustes Analysis (GPA) was employed to minimize the disparities in scores assigned by the panellists to five specific traits that were found to be significantly influenced by the sample type. The objective was to reach a consensus and present the findings in a biplot map representing the attributes and samples. This methodology was described by Pateiro et al. (2022).

4.8. Sensory Analysis

A panel of 15 assessors, consisting of students and staff from the Faculty of Tropical AgriSciences at the Czech University of Life Sciences Prague, was assembled to conduct a descriptive assessment of the product (Figure 9). The assessors underwent training on how to assess the sensory characteristics and how to complete the evaluation framework. A non-structured graphical 100 mm scale, composed of straight lines aligned with descriptions at both ends, was employed for the purpose of sensory evaluation and rating. The samples were prepared one day in advance and stored in sealed plastic bags to preserve the Thyme and Ajwain flavour. In addition, during the salting process, the meat sample was immersed in a salt solution containing 12 grams of NaCl per liter for a duration of ten minutes. The meat was then dried using the same method as stated before in the preparation of the modified blanching treatments.

Figure 10. Students and staff from CZU-FTA participated in sensory analysis

The samples were encoded using randomly generated three-digit numbers. Each panellist was provided with six samples—(1) control + salt (2) Ajwain OT 0.75 mL (3) Thyme HAB 0.75 mL (4) Ajwain HAB 0.75 mL (5) Thyme OT 1.5 mL and (6) Ajwain OT 1.5 mL. In order to cleanse the assessor's taste, unsalted bread, water, and Ethanol 30% were provided after each sample (Figure 10).

Figure 11. Layout of sensory analysis for each participant

The factors that were assessed were the visual appearance, aroma, colour, texture, taste, intensity of individual flavours, and overall evaluation of the jerky samples (Table 1). The sensory analysis was conducted over two weeks, consisting of two sessions, in the laboratory of sensory analysis in Faculty of Tropical AgriSciences at Czech University of Life Sciences-Prague.

Table 1. Sensory traits and their scale orientations

Sensory trait	Scale orientation	
	left = 0 %	right = 100 %
Appearance:		
general appearance	very bad	excellent
Smell:		
general pleasantness of the smell	very bad	excellent
intensity of thymol smell	imperceptible	very strong
Colour:		
general likableness of the colour	dislike	like
general intensity of the colour	extremely light	extremely dark
Texture:		
general pleasantness of the texture	very bad	excellent
juiciness	dry	juicy
chewiness	difficult	easy
Taste:		
general pleasantness of the taste	very bad	excellent
general intensity of the taste	imperceptible	very strong
Intensity of partial tastes:		
Thymol taste	imperceptible	very strong
salty	imperceptible	very strong
bitter	imperceptible	very strong
astringent	imperceptible	very strong
pungent	imperceptible	very strong
Overall evaluation of the sample:	very bad	excellent

5. Results

5.1. Identification of volatile compounds

Table 2 displays the findings of the chemical composition analysis conducted on Thyme essential oil (TEO) and Ajwain essential oil (AEO). There were 10 components discovered for pure TEO, which accounted for 95.15 % of the total oil area. The highlighted compounds under TEO consisted primarily of β-Pinene, *p*-Cymene, γ-Terpinene, and Thymol. The primary constituent was Thymol, accounting for 46.98 % of the molecule. It was accompanied by three monoterpene hydrocarbons: γ-Terpinene (26.78 %), *p*-Cymene (18.32 %), and β-Pinene (1.89 %). Simultaneously, 11 compounds were found for AEO, together accounting for 99.99 % of the entire oil area. The four highlighted compounds under Ajwain essential oil (AEO) contained β-Pinene, *p*-Cymene, γ-Terpinene, and Thymol as its main components. The main constituent was Thymol, accounting for 37.03 % of AEO. Additionally, γ-Terpinene made up 35.75 %, *p*-Cymene accounted for 23.67 %, and β-Pinene constituted 2.24 %.

Table 2. Principal constituents of Thyme and Ajwain essential oils and their relative percentages of total Chromatogram area and Retention indices.

Compounds	RI Lit	RI	Area %	
			Ajwain	Thyme
α-Thujene	931	924	0.2	0.18
α-Pinene	937	930	0.3	0.26
β-Pinene	**980**	**975**	**2.24**	**1.89**
β-Myrcene	992	990	0.33	0.32
α-Terpinene	1018	1016	0.27	0.27
***p*-Cymene**	**1027**	**1027**	**23.67**	**18.32**
γ-Terpinene	**1062**	**1062**	**35.75**	**26.78**
Isoterpinolene	1086	1090	0.04	-
p-Cymenene	1090	1093	0.07	0.08
Limonene oxide	1183.9	1172	0.09	-
Thymol	**1290**	**1315**	**37.03**	**46.98**
Carvone	1242	-	-	0.07

[a] Compound identification was based on the Retention index (RI) and comparison with the NIST database. [b] KI Adam's library. [c] RI (Ramos et al., 2000)

Figure 12 shows the chromatograms of Ajwain essential oil and Thyme essential oils where it demonstrated almost identical content of volatile compounds.

In Table 3 presents the mean composition percentages and accompanying Standard Deviation (SD) at a significant value of ($p < 0.05$) for two essential oils, two modified blanching treatments, two doses, and the four volatile compounds namely β-Pinene, *p*-Cymene, γ-Terpinene, and Thymol following meat drying. The findings demonstrated notable disparities in the composition of the treated and control samples.

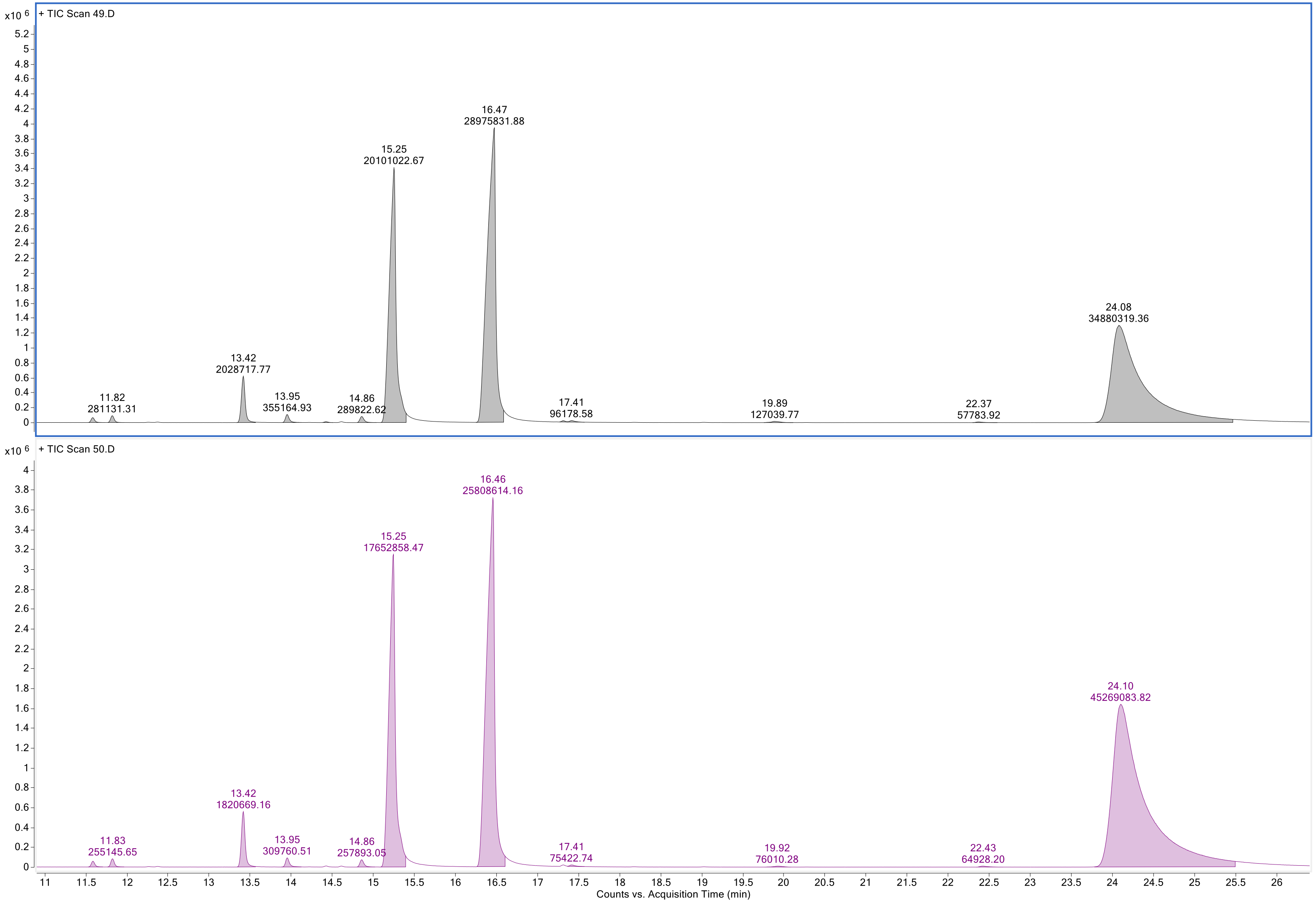


Figure 12. Chromatograms of Ajwain essential oil (top) and Thyme essential oil (bottom)

For β-Pinene, it was found that Thyme OT 1.5 mL has the most content (2.66 ± 1.19 %) among other samples. In addition, there are no significant differences found between Thyme control, Ajwain control, Ajwain HAB 0.75 mL, and Thyme HAB 0.75 mL samples but are found to be significantly different to Ajwain OT 0.75 mL. Thyme OT 0.75 mL and Thyme OT 1.5 mL was also found not to be significant different from each other. On the other hand, it was found that Ajwain HAB 1.75 mL and Ajwain OT 0.75 mL are significantly different to majority of the samples when it comes to β-Pinene area %.

The area % of *p*-Cymene of Ajwain HAB 1.5 mL was found to be significantly different among the rest of the samples. Thyme HAB 1.5 mL, Thyme OT 0.75 mL, Ajwain OT 1.5 mL samples are found to be not significantly different to each other. Thyme OT 1.5 mL, Ajwain HAB 0.75 mL, Ajwain OT 0.75 mL are also not significantly different to each other, however, significantly different from the remainder of the samples.

In γ-Terpinene, the area % of samples Thyme OT 0.75 mL, Ajwain HAB 0.75 mL and Ajwain OT 0.75 mL has no significant difference between each other, however, they are found to be significantly different among the rest of the samples. Ajwain HAB 1.5 mL sample is found to be significantly different to Ajwain control sample.

The area % of Thymol of Thyme and Ajwain control samples are found to be not significantly different, but both samples are significantly different to the rest of treated dried meat samples. In addition, Thyme OT 0.75 mL, Ajwain HAB 0.75 mL, and Ajwain HAB 1.5 mL are found to be not significantly different, however, significantly different to samples such as Thyme HAB 0.75 mL, Thyme HAB 1.5 mL, Thyme OT 1.5 mL, and Thyme and Ajwain controls. The same results also occurred for samples Thyme HAB 1.5 mL and Thyme OT 1.5 mL which are not significantly different to each other, but are significantly different to Thyme and Ajwain controls, Thyme OT 0.75 mL, Ajwain HAB 0.75 mL, Ajwain HAB 1.5 mL, Ajwain OT 0.75 mL, and Ajwain OT 1.5 mL samples.

Figure 13 portrays the chromatograms and relative peak areas for the observed volatile compounds in dried meat samples of Thyme HAB 0.75 mL (22D), Thyme OT 0.75 mL (23D), Thyme OT 1.5 mL (35D), and Thyme HAB 1.5 mL (25D). Subsequently, Figure 14 shows the chromatograms and relative peak areas of observed volatile compounds in Ajwain dried meat samples namely, Ajwain HAB 0.75 mL (27D), Ajwain HAB 1.5 mL (28D), Ajwain OT 0.75 mL (31D), and Ajwain OT 1.5 mL (37D).

Table 3. Quantification in mean (area %) +/- standard deviation (SD) of the principal constituents for the different treatments and essential oil doses in dried meat samples

Treatment type			Mean ± SD of Area % (n=6)			
Essential oil	Treatment	Dose (mL)	ß-Pinene	p-Cymene	γ-Terpinene	Thymol
Thyme	Control	0	0.04 ± 0.09^{a}	7.72 ± 4.36^{a}	3.65 ± 2.03^{ab}	1.42 ± 1.49^{a}
Thyme	HAB	0.75	0.22 ± 0.12^{a}	11.55 ± 6.28^{ab}	4.28 ± 2.55^{ab}	12.87 ± 3.80^{e}
Thyme	HAB	1.5	0.63 ± 0.13^{ab}	25.09 ± 5.42^{c}	11.73 ± 3.33^{bcd}	11.89 ± 3.91^{de}
Thyme	OT	0.75	2.29 ± 1.44^{cd}	24.53 ± 8.75^{c}	9.45 ± 4.85^{abcd}	5.93 ± 1.90^{bc}
Thyme	OT	1.5	2.66 ± 1.19^{cd}	19.94 ± 6.04^{bc}	7.08 ± 1.95^{abc}	11.63 ± 5.41^{de}
Ajwain	Control	0	0.10 ± 0.16^{a}	7.05 ± 8.36^{a}	1.83 ± 2.44^{a}	0 ± 0^{a}
Ajwain	HAB	0.75	0.46 ± 0.09^{a}	20.67 ± 5.15^{bc}	9.59 ± 2.48^{abcd}	6.66 ± 1.72^{bc}
Ajwain	HAB	1.5	1.14 ± 0.55^{abc}	40.84 ± 6.91^{d}	16.15 ± 7.66^{d}	7.81 ± 0.85^{bc}
Ajwain	OT	0.75	1.75 ± 0.63^{bcd}	20.82 ± 2.48^{bc}	8.84 ± 2.98^{abcd}	5.26 ± 1.30^{b}
Ajwain	OT	1.5	2.79 ± 0.38^{d}	27.47 ± 5.48^{c}	12.52 ± 4.56^{cd}	8.82 ± 2.19^{cd}

Note: HAB = Hot air blanching; OT = Oil Treatment

Values are given as mean ± SD (n=12). Values represent means of two replicates/trials. Values in the same column followed by different high case letters are significantly different at $p < 0.05$.

Figure 15 shows β-Pinene area % of samples with different blanching methods at different doses. It shows that Thyme HAB, Ajwain HAB, and Ajwain OT samples with 0.75 mL concentration contain 0.22 ± 0.12 %, 0.46 ± 0.49 %, and 1.75 ± 0.63 %, respectively. It was observed that the means is at a higher amount of β-Pinene content at a higher dose. At 1.5 mL concentration, Thyme HAB, Ajwain HAB, and Ajwain OT obtained 0.63 ± 0.13 %, 1.14 ± 0.55 %, and 2.79 ± 0.38 %, respectively. On the other hand, β-Pinene content for Thyme OT decreased from 2.29 ± 1.44 % to 1.30 ± 0.68 % at an incresased dose. For control samples of Ajwain and Thyme, it was found an area β-Pinene (%) of 0.10 ± 0.16 and 0.04 ± 0.09, respectively.

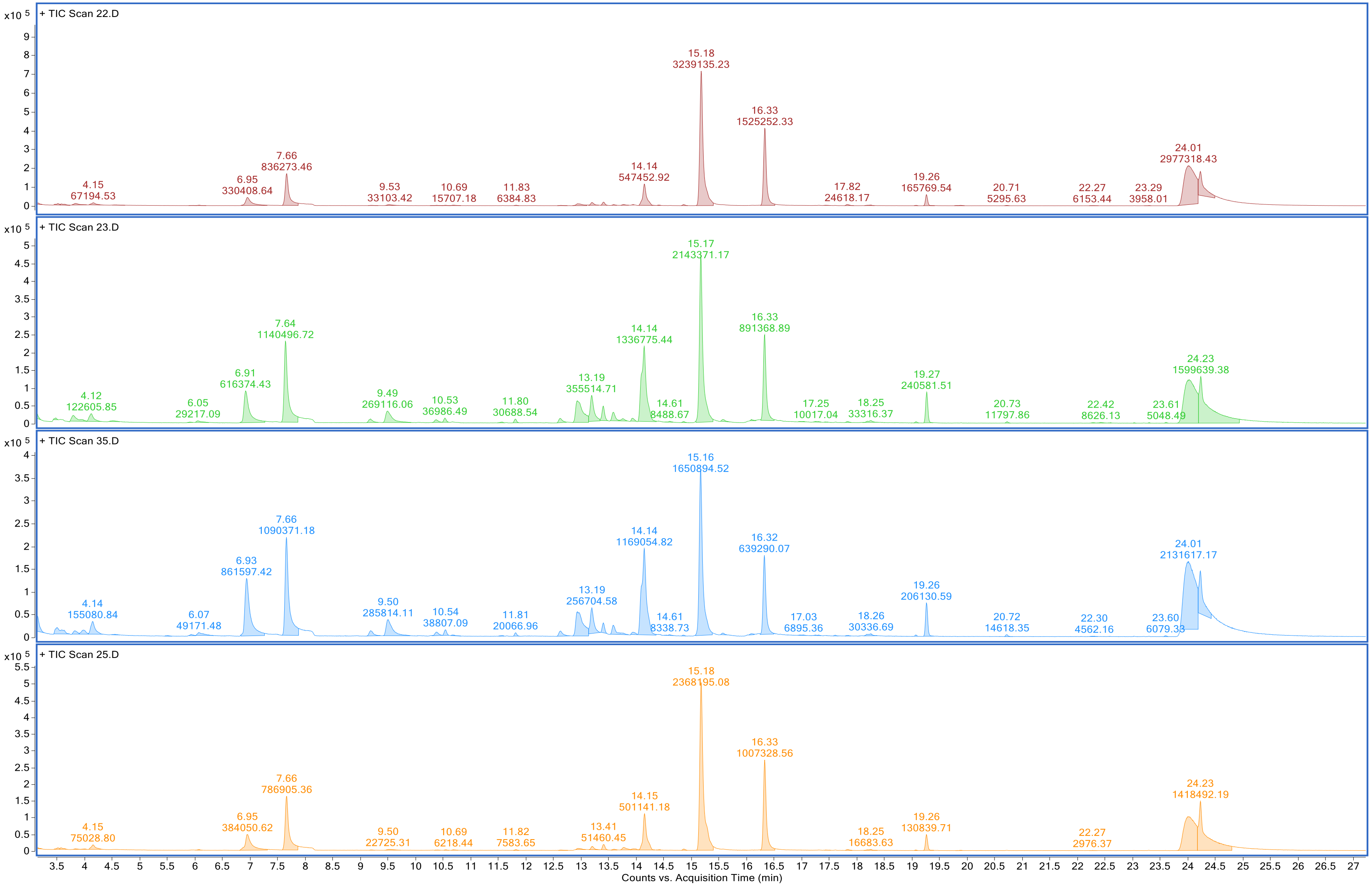


Figure 13. Chromatograms of THAB 0.75 mL sample, TOT 0.75 mL sample, TOT 1.5 mL sample, THAB 1.5 mL dried meat samples (top to bottom)

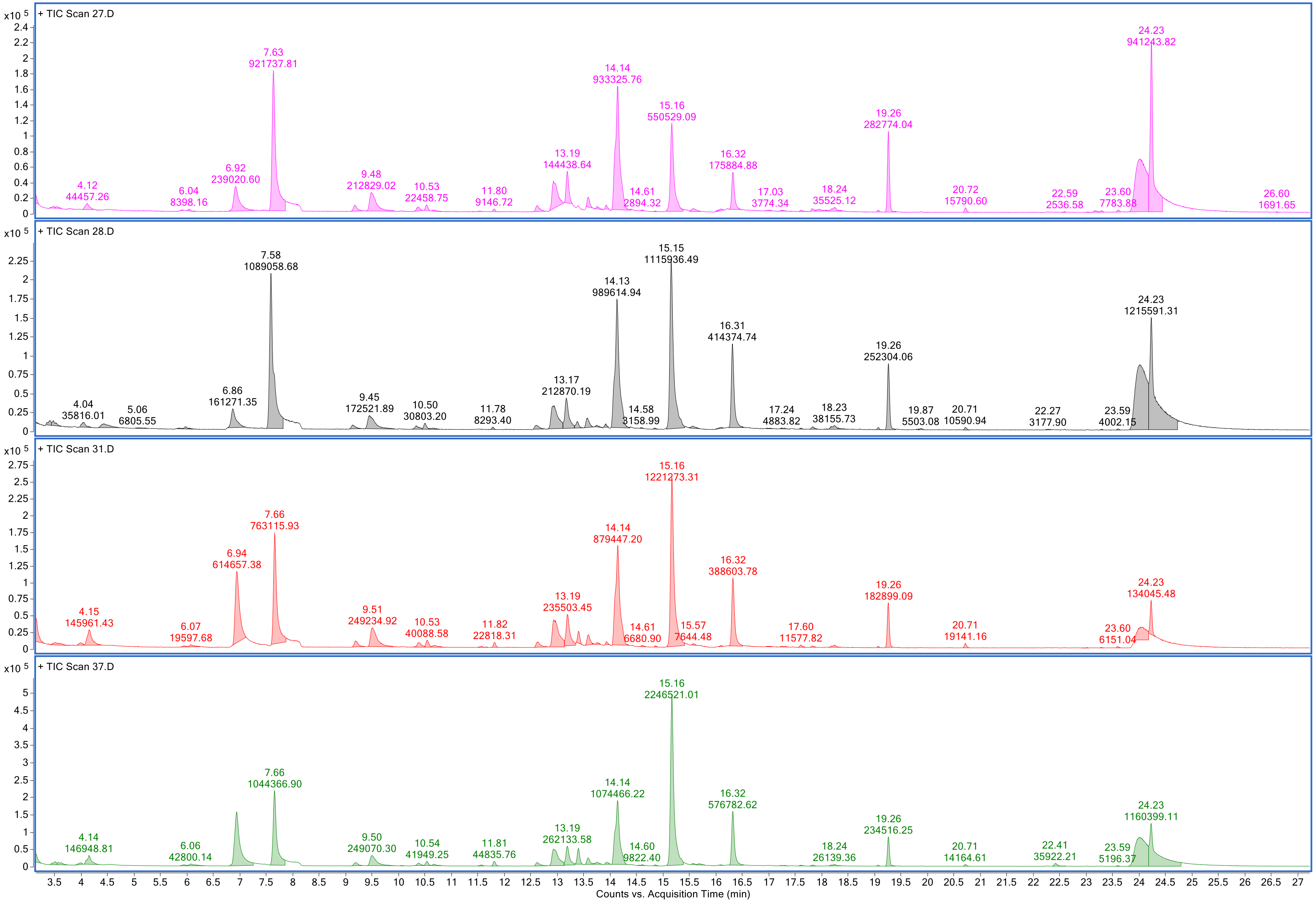


Figure 14. Chromatograms of AHAB 0.75mL, AHAB 1.5 mL, AOT 0.75 mL, and AOT 1.5 mL dried meat samples (top to bottom)

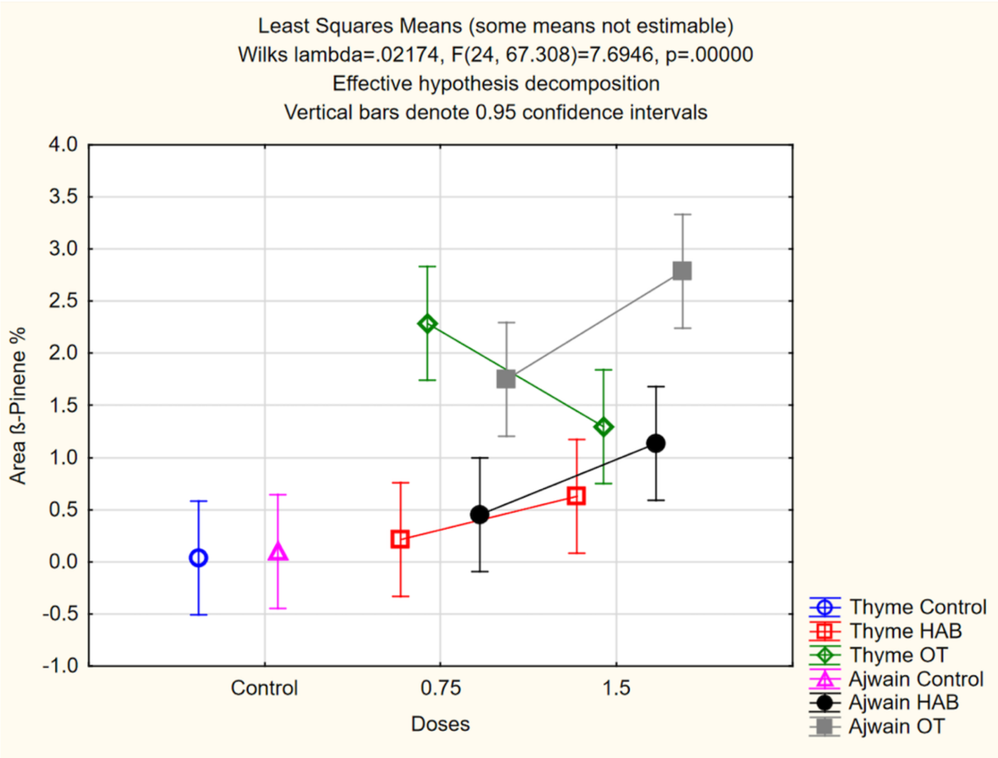


Figure 15. Analysis of variance of treated samples and their Area β-Pinene (%) content in mean (standard deviation)

The area γ-Terpinene (%) of samples exhibited similar behavior as area β-Pinene except for the control samples, as shown in Figure 16. The Thyme control obtained 3.65 ± 2.03, and the Ajwain control obtained 1.83 ± 2.44.

Meanwhile, the area γ-Terpinene (%) of Thyme HAB, Ajwain HAB, and Ajwain OT is 4.28 ± 2.55, 9.59 ± 2.48, and 8.84 ± 2.98 at a 0.75 mL concentration. This component significantly increased as the dose increased, at values of 11.73 ± 3.33, 16.15 ± 7.66, and 12.52 ± 4.56. However, the same pattern occurred for Thyme OT, in which γ-Terpinene content decreased from 9.45 ± 4.85 to 7.08 ± 1.95 as the dosage increased.

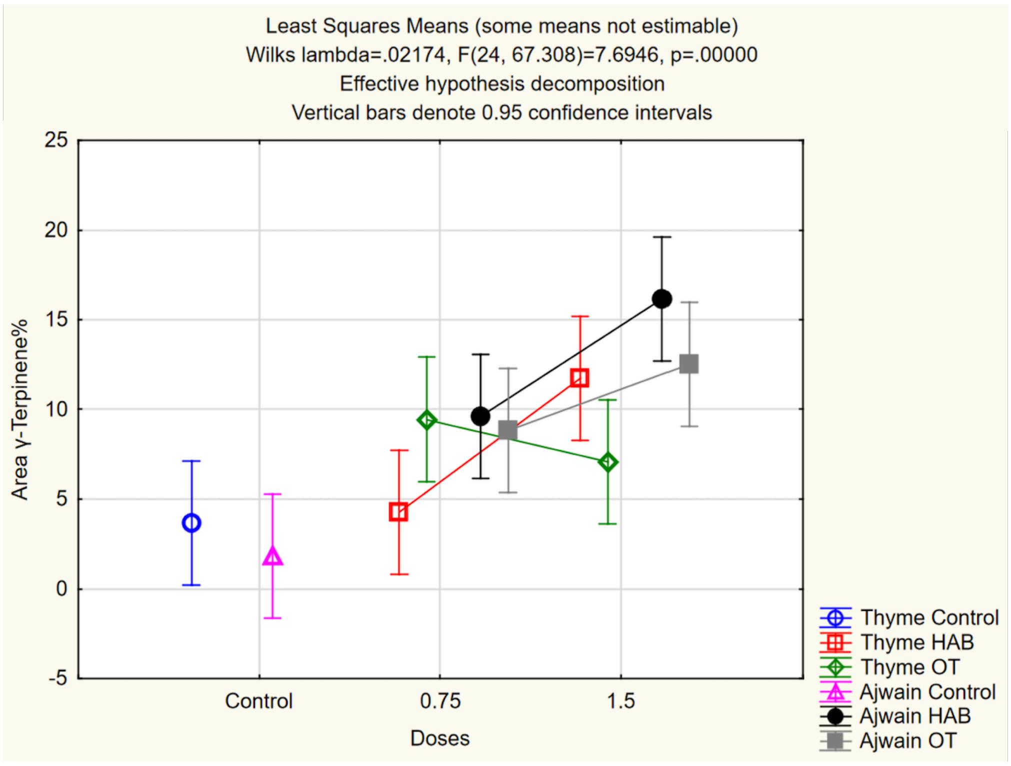


Figure 16. Analysis of variance of treated samples and their Area γ-Terpinene (%) content in mean (standard deviation)

In Figure 17, the area thymol % of Thyme OT, Ajwain HAB, and Ajwain OT samples showed an increasing pattern as the dosage increased. It increased from 0.75 mL dose with values of 5.93 ± 1.90, 6.66 ± 1.72, and 5.26 ± 1.30 to 11.63 ± 5.41, 7.81 ± 0.85, and 8.82 ± 2.19 at 1.5 mL dose, respectively. However, Thyme HAB showed a declining pattern from 12.87 ± 3.80 to 11.89 ± 3.91 as the dosage went higher. For control samples, Thyme control (1.42 ± 1.49) and Ajwain control (0.00 ± 0.00), the pattern is almost similar to the controls of γ-Terpinene.

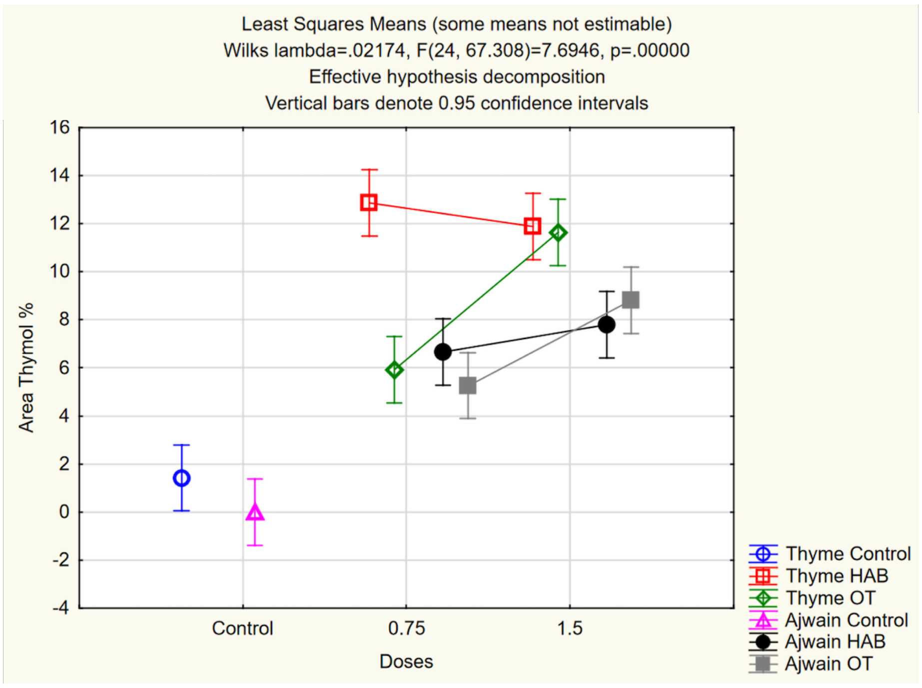


Figure 17. Analysis of variance of treated samples and their Area Thymol (%) content in mean (standard deviation)

The Thyme OT sample decreased from a value of 24.53 ± 8.75 to 19.94 ± 6.04 as the dose went higher (Figure 14). This same pattern of area *p*-Cymene % was also observed in the area β-Pinene % and area γ-Terpinene %.

On the other hand, the Thyme HAB, Ajwain HAB, and Ajwain OT samples obtained an area *p*-Cymene % of 11.55 ± 6.28, 20.67 ± 5.15, and 20.82 ± 2.48 at a 0.75 mL dose. Following this, the samples' area *p*-Cymene % composition went up to 25.09 ± 5.42, 40.84 ± 6.91, and 27.47 ± 5.48 as the dosage escalated to 1.5 mL, respectively. The controls of Ajwain and Thyme had traces of *p*-Cymene (7.05 ± 8.36 and 7.72 ± 4.36), likely due to the presence of volatile compounds in the surroundings.

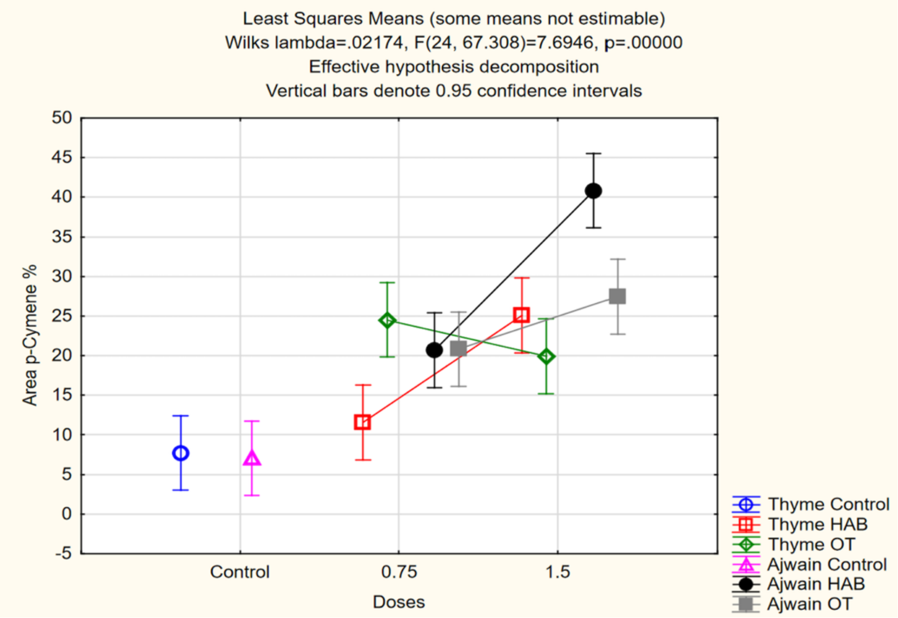


Figure 18. Analysis of variance of treated samples and their Area p-Cymene (%) content in mean (standard deviation)

5.2. Sensory Analysis

The results of sensory analysis were separated into two tables, Table 4 and Table 5. Table 4 shows the means (standard deviation) of general sensory attributes. On the other hand, Table 5 displays the means (standard deviation) obtained from sensory evaluation of attributes of the samples according to taste.

To investigate the primary sensory qualities of dried meat, we analyzed 16 attributes. Six samples were selected for the sensory analysis as it is recommended in ISO 8587:2006-- that a maximum of around 6-7 samples are enough for sensory evaluation to avoid overworking the taste buds of the evaluators. The following six samples were (1) control + salt, (2) Thyme HAB 0.75 mL, (3) Ajwain HAB 0.75 mL, (4) Ajwain OT 0.75 mL, (5) Thyme OT 1.5 mL, and (6) Ajwain OT 1.5 mL. The ANOVA main effects analysis revealed significant differences in 5 of the 16 sensory qualities tested, as shown in Tables 4 and 5. These five sensory qualities are intensity of the thymol smell, general intensity of the colour, general intensity of the taste, thymol taste, and bitter. The results of the remaining 11 sensory attributes were found to be not significantly different.

The sample control + salt was shown to be significantly different from other samples except for Ajwain HAB 0.75 mL, in terms of the intensity of the thymol smell. However, it was found that there is a significant difference found between control + salt sample to other samples except for Ajwain HAB 0.75 mL sample. Regarding the general intensity of the colour of the samples, it was found that the control sample with salt was significantly different from the others. Thyme HAB 0.5 mL, Ajwain HAB 0.75 mL, and Ajwain OT 1.5 mL samples does not have significant difference among each other, but these samples are found to be significantly different to control + salt, Ajwain OT 0.75 mL, and Thyme OT 1.5 mL.

Table 4. Mean (standard deviation) sensory trait scores (%) of dried meat sample

Sensory Attribute	Control + salt	Thyme HAB 0.75 mL	Ajwain HAB 0.75 mL	Ajwain OT 0.75 mL	Thyme OT 1.5 mL	Ajwain OT 1.5 mL
General Appearance	51.07 (20.32)^{a}	51.33 (23.17)^{a}	53.00 (22.95)^{a}	59.80 (27.45)^{a}	63.73 (19.78)^{a}	52.77 (22.68)^{a}
General pleasantness of the smell	51.90 (10.26)^{a}	50.60 (19.90)^{a}	44.03 (14.84)^{a}	56.00 (20.20)^{a}	55.00 (24.47)^{a}	48.93 (24.57)^{a}
Intensity of the thymol smell	19.13 (18.08)^{a}	45.13 (18.94)^{b}	30.27 (26.96)^{ab}	50.47 (17.83)^{b}	49.90 (23.64)^{b}	45.07 (21.11)^{b}
General likableness of the colour	51.13 (22.81)^{a}	54.67 (22.93)^{a}	45.10 (29.90)^{a}	66.53 (24.82)^{a}	67.47 (24.68)^{a}	61.67 (24.97)^{a}
General intensity of the colour	45.47 (12.59)^{a}	55.07 (17.66)^{ab}	51.37 (24.43)^{ab}	66.07 (18.90)^{b}	66.13 (16.70)^{b}	49.60 (22.37)^{ab}
General pleasantness of the texture	46.23 (17.33)^{a}	45.53 (20.32)^{a}	49.00 (25.28)^{a}	50.30 (19.80)^{a}	49.40 (25.87)^{a}	56.80 (19.84)^{a}
Juiciness	27.93 (20.35)^{a}	33.20 (23.64)^{a}	29.80 (23.73)^{a}	35.10 (21.43)^{a}	39.33 (30.01)^{a}	42.87 (22.32)^{a}
Chewiness	51.60 (20.66)^{a}	52.77 (19.91)^{a}	44.27 (19.24)^{a}	58.33 (21.13)^{a}	54.20 (27.45)^{a}	60.60 (21.24)^{a}

Means in the same row followed by different superscript letters are significantly different ($P < 0.05$). For the evaluation, unstructured graphical 100 mm linear scales were used. The general appearance, the general pleasantness of the smell, and the texture (0% = very bad and 100% = excellent). The intensity of thyme smell (0% = imperceptible and 100% = very strong). The general likableness of the colour (0% = dislike and 100% = like) and general intensity of the colour (0% = extremely light and 100% = extremely dark). Juiciness and chewiness (0% = dry, difficult and 100% = juicy, easy).

In Table 5, control + salt sample was found to be significantly different from Thyme OT 1.5 mL and Ajwain OT 0.75 mL in terms of the sensory attribute such as general intensity of the taste. On the other hand, Thyme HAB 0.75 mL, Ajwain HAB 0.75 mL, and Ajwain OT 1.5 mL were found to be not significantly different from each other, however, significantly different to Ajwain OT 0.75 mL, Thyme 1.5 mL, and control + salt samples. In thymol taste, it was found that control + salt sample is significantly different from the other samples.

Table 5. Mean (standard deviation) sensory trait scores for taste (%) of dried meat sample

Sensory Attribute	Control + salt	Thyme HAB 0.75 mL	Ajwain HAB 0.75 mL	Ajwain OT 0.75 mL	Thyme OT 1.5 mL	Ajwain OT 1.5 mL
General pleasantness of the taste	48.33 (17.48)^{a}	42.53 (23.82)^{a}	44.47 (22.11)^{a}	56.53 (19.59)^{a}	48.53 (26.02)^{a}	50.53 (22.38)^{a}
General intensity of the taste	39.73 (19.98)^{a}	52.40 (16.96)^{ab}	56.33 (17.74)^{ab}	62.40 (16.43)^{b}	58.73 (15.62)^{b}	57.33 (15.62)^{ab}
Thymol taste	23.40 (19.79)^{a}	55.27 (16.68)^{b}	56.27 (19.69)^{b}	57.33 (17.83)^{b}	51.97 (17.22)^{b}	47.00 (16.96)^{b}
Salty	41.40 (16.72)^{a}	29.13 (16.15)^{a}	31.93 (22.18)^{a}	34.53 (19.04)^{a}	37.13 (12.51)^{a}	35.30 (17.48)^{a}
Bitter	12.00 (15.15)a	31.60 (27.23)^{ab}	33.00 (23.03)^{ab}	23.33 (21.47)^{ab}	39.20 (28.27)^{b}	29.63 (24.12)^{ab}
Astringent	18.40 (16.37)^{a}	28.20 (25.72)^{a}	29.23 (27.14)^{a}	21.73 (20.38)^{a}	39.33 (30.32)^{a}	28.73 (26.26)^{a}
Pungent	18.73 (18.16)^{a}	28.53 (24.27)^{a}	28.06 (27.06)^{a}	26.23 (21.84)^{a}	27.43 (24.85)^{a}	23.33 (21.66)^{a}
Overall evaluation of the sample	50.70 (17.19)^{a}	46.60 (19.60)^{a}	45.07 (24.51)^{a}	52.80 (17.92)^{a}	48.80 (25.15)^{a}	54.33 (17.55)^{a}

Note: Means in the same row followed by different superscripts letters are significantly different ($P < 0.05$). For the evaluation, unstructured graphical 100 mm linear scales were used. The overall evaluation and the general pleasantness of the taste (0% = very bad and 100% = excellent). General intensity of taste, and intensity of partial tastes: thyme taste, salty, bitter, astringent, and pungent (0% = imperceptible and 100% = very strong).

The remaining samples did not have significant difference among each other. In bitter taste, control + salt sample and Thyme OT 1.5mL are found to be significantly different to each other, but both are not significantly different to samples Thyme HAB 0.75 mL, Ajwain HAB 0.75 mL, Ajwain OT 0.75 mL, and Ajwain OT 1.5 mL samples. On the overall evaluation of the samples, Ajwain OT 1.5 mL was rated to be the most excellent among the rest. Although, generally, there are no significant differences found among each other.

In Figure 19, it shows the Generalized Procrustes Analysis (PCA) of the sensory analysis conducted for the selected samples. We chose five sensory traits as the criteria based on the results previously evaluated using ANOVA, taking into account the impact of the sample. The F1 and F2 dimensions derived from the GPA accounted for 73.89 % of the overall variability, as seen in Figure 19. Based on the findings, the taste of Thyme HAB 0.75mL was primarily characterized by the presence of Thymol. The intensity of the taste and colour was influenced by the addition of Ajwain OT 0.75 mL. Additionally, the intensity of the Thymol smell was affected by the presence of both Thyme and Ajwain OT 1.5 mL. However, it is important to note that the overall impact of these criteria on the control and Thyme HAB 0.75 mL samples was minimal.

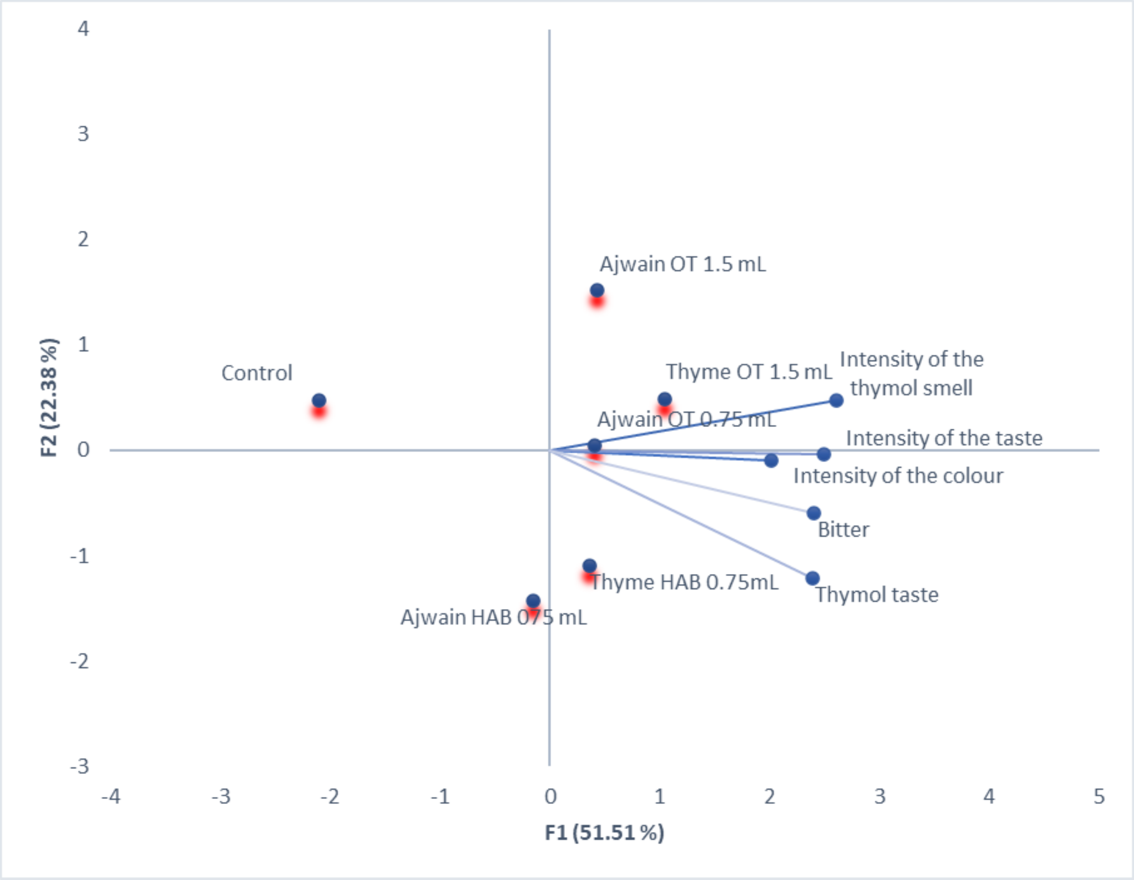


Figure 19. GPA biplot of attributes and samples.
Note: the sensory qualities are represented by blue dots. Treatments are depicted by blue and red circles.

6. Discussion

6.1. Identification of volatile compounds

Thymol, *p*-cymene, and γ-terpinene are important components of the essential oils of *Thymus vulgaris* and *Trachyspermum ammi L*, as described by Horváth et al. (2006), Stahl-Biskup (2003), Bairwa et al. (2012), and Gaba et al. (2018). The monoterpenes found in TEO are consistent with those documented by Viuda-Martos et al. (2010). The essential oil of *T.vulgaris* from Egypt, taken from the complete plant including stems, leaves, and flowers, was found to include thymol (32.23 %), γ-terpinene (21.19 %), and *p*-cymene (20.27 %) as its primary constituents. Furthermore, these findings align with the results previously documented in Romania (Jianu 2014), which identified the primary constituents of the *T.vulgaris* essential oil (obtained from the aboveground portion of the plant) as thymol (47.59 %), γ-terpinene (30.90 %), and *p*-cymene (8.41 %)

The findings of our study on the composition of *Trachyspermum ammi L* were consistent with the findings of Zarshenas et al. (2014) and Khan et al. (2020). Based on their results, the major compounds reported were Thymol, γ-Terpinene, *p*-Cymene, and *β*-pinene. In both studies, the largest component identified was Thymol, which accounted for 35.04 % of the composition. The content of γ-Terpinene was 23.11 %, while the content of *p*-Cymene was 31.80 %. While other research indicates that γ-Terpinene and *p*-Cymene had higher levels of Thymol content in North Sinai, Egypt, it is concluded that the plant was grown in soil with a higher saline concentration (Omer et al. 2014).

In this study, we chose β-Pinene, *p*-Cymene, γ-Terpinene, and Thymol as the primary components for our analysis because they remained the predominant components even after the application of the EOs treatments. Higher doses of OT samples containing β-Pinene were reported to be greater (P<0.05) than the control and HAB samples. Ahmed et al. (2019) also found the same high concentration of β-Pinene as this study result shown. The β-Pinene was observed to be the greatest in OT 1.5 mL (2.66 %) of both essential oils. This discrepancy was primarily due to the fact that dipping in sunflower oil may have increased the proportion of certain monoterpene hydrocarbons while accelerating the oxidation process.

Ajwain HAB 1.5 mL contained a considerably higher concentration of *p*-Cymene (40.84 %) than the control and OT samples. It is noteworthy that the *p*-Cymene content in purified AEO is around 23.67 % (Table 2), which is nearly double the value (Table 3). Given that *p*-Cymene can be generated via isomerisation and oxidation from monoterpene hydrocarbons, it is conceivable that this pathway could be utilised to produce this class of aromatic compounds, with its quantity potentially increasing throughout thermal processing (Heatherbell et al. 1971; Mahanta et al. 2021).

The primary samples with Ajwain HAB 1.5 mL (%), Thyme HAB 1.5 mL (%), and Ajwain OT 1.5 mL (%) had a substantially higher share of γ-terpinene (P<0.05) compared to the control (Table 3). Furthermore, it is worth noting that the γ-Terpinene content was comparatively lower than the initial proportions found in Thyme and Ajwain essential oils (26.78 and 35.75 %, respectively). This outcome could potentially be attributed to the application of heat through. Heat plays a significant role in metabolite degradation, which is accelerated at higher temperatures. Degradation can proceed through many mechanisms, including oxidative degradation, C-C bond cleavage, elimination, hydrolysis, and thermal rearrangement (Mahanta et al. 2021). Jakab et al. (2018) also observed the synthesis of *p*-cymene and *p*-cymenene from monoterpenes like limonene, α-phellandrene, γ-terpinene, and α-terpinene.

Furthermore, the phenolic content, specifically Thymol, which is indicative of the grade of Ajwain and Thyme oil, was found to be higher in OT and HAB samples administered at the higher dose (1.5 mL) (Table 3). It is significantly greater in Thyme HAB 0.75 mL (12.87 %) and Ajwain OT 1.5 mL (8.82 %). While the mean concentrations of Thymol in the various treatments are representative, the proportion was significantly lower in comparison to the Thymol found in the purified essential oils (Table 2). One possible explanation is that although the concentrations of γ-Terpinene and *p*-Cymene, which are precursors in the biosynthesis of Thymol, increased, those of Thymol decreased (Condurso et al., 2013; Gomori et al., 2018).

6.2. Sensory Analysis

Visual appearance, as well as in-mouth perceptions of flavour and texture, are among the most important sensory characteristics impacting consumer perceptions of meat products (Bredahl 2004; Grunert et al. 2004; Morales et al. 2007; Font-i-Furnols & Guerrero 2014). Moreover, Chivandi et al. (2016) reported essential oils possess a powerful odour that can be overpowering for consumers, even when used in little amounts. Applying EOs directly to meat on a wide scale to properly preserve it would be challenging and expensive due to the need to ensure controlled and sufficient levels of EOs come into contact with the meat. Consequently, conducting a sensory evaluation would be useful to assess the consumer perception towards meat applied with EOs. The methods for determining the general qualities of appearance, smell, colour, and texture are presented in Table 4.

When comparing the average values of several samples, the panelists determined that Ajwain OT 0.75 mL had a more pleasant smell overall and a stronger Thyme scent compared to the control ($P<0.05$). In terms of colour preference, samples treated with OT received higher scores compared to both the HAB samples and the control group. The OT samples' increased acceptance is connected with their intense dark reddish color and polished appearance. Font-i-Furnols & Guerrero (2014) found that consumers associate red-purple hues with freshness and brown colours with a lack of brightness. This study observed a similar pattern. Simultaneously, the outcomes derived from overall appearance indicated that the highest scoring samples were those containing 0.75 mL of Ajwain and 1.5 mL of Thyme. According to the literature, the colour of meat products has an impact on their overall look and can influence how consumers perceive their quality.

Typically, texture and flavour are recognized as the primary indicators of beef quality. In terms of texture, the ajwain OT 1.5 mL sample was found to be superior to the other samples in terms of overall pleasantness of texture, juiciness, and chewiness. The samples with 0.75 mL of Ajwain and 1.5 mL of Thyme were deemed satisfactory in terms of the overall pleasantness of the texture, as compared to other samples and the control. Additionally, they were rated as not tough and less intense in terms of juiciness. Nevertheless, no notable disparities were found amongst the samples in terms of texture. Consumers likely anticipate and tolerate a certain level of firmness in cured meat as opposed to fresh meat. Thus, the evaluation of texture attributes appears to be more challenging compared to the assessment of smell and taste in relation to these specific items (Sañudo et al., 2016).

The results from the evaluation of sensory attributes, such as taste and intensity of taste, are displayed in Table 5.. Ajwain OT 0.75 mL exceeded the control and other samples in terms of the overall pleasantness and intensity of the flavour. Simultaneously, it was scored as the most concentrated in Thymol flavour as compared to the control, aligning with our earlier findings regarding the intensity of scent (Table 4). Chivandi et al. (2016) emphasized similar observation wherein essential oils possess intense scent that may influence the intensity of taste and sensory perception of consumers on the food product. With respect to the salty flavour (Table 5), the samples exhibited no notable variations and were classified as lacking in saltiness. The control was viewed as the most salty; it is most probable that the absence of Thymol enables other subtle flavours to dominate. Another study by Escobar et al. (2020) confirmed that a certain level of concentration from essential oils can affect the natural flavour of the dish.

Regarding bitterness, there were significant variations seen among the samples ($P<0.05$). Thyme OT 1.5 mL had the highest level of bitterness in comparison to the control. The examination does not reveal any notable variations in terms of astringent and pungent characteristics across the meat samples, as indicated in Table 5. Although there was no significant difference ($P<0.05$) in taste intensity for Ajwain OT doses of 0.75 mL and 1.5 mL, the tendency indicates that increasing the EO dose often leads to a higher taste magnitude, typically resulting in a more bitter taste as also reported by Amiri et al. (2024).

We used a Generalized Procrustes Analysis (PCA) to mitigate the influence of scale variations and achieve a consensus among panellists about the ratings assigned to some crucial sensory qualities (Vargas-Ramella et al., 2021). Ultimately, the Ajwain OT samples of 0.75 mL and 1.5 mL demonstrated higher performance in the entire assessment of the sample, as indicated in Table 5. The qualities of smell, colour, and taste were found to have a significant impact on the overall rating score in this study. In contrast to other research, where variations in texture may impact the overall perceived quality (Bello & Calvo 2000; Morales et al. 2008).

7. Conclusion

Essential oils have significant potential to enhance the sensory attributes and nutritional value of dried meat. The HS-SPME technique, which does not require a solvent, when paired with GC/MS analysis, provides a valuable option for extracting, identifying, and quantifying volatile chemicals. The four main monoterpene compounds, namely thymol, γ-Terpinene, *p*-Cymene, and β-Pinene, were detected in both Ajwain essential oil and Thyme essential oil using HS-SPME-GC/MS. The percentages of these compounds in Ajwain essential oil were as follows: Thymol (37.08 %), γ-Terpinene (35.75 %), *p*-Cymene (23.67 %), and β-Pinene (2.24 %). In Thyme essential oil, the percentages were: Thymol (46.98 %), γ-Terpinene (26.78 %), *p*-Cymene (18.32 %), and β-Pinene (1.89 %). The bioactive chemicals, which possess antibacterial, antioxidant, and other therapeutic characteristics, were also employed as a natural preservative for dried meat. The dried meat samples, which were subjected to different doses and procedures, displayed distinct trends for each identified volatile component. OT samples exhibited elevated amounts of β-Pinene compared to HAB samples and controls. The Ajwain HAB 1.5 mL sample has the greatest *p*-Cymene level compared to the other samples. However, the samples with the highest γ-Terpinene content include Ajwain HAB 1.5 mL, Thyme HAB 1.5 mL, and Ajwain OT 1.5 mL. Thymol concentration was observed to be greater in samples with a higher dosage (1.5 mL) of essential oil.

During the sensory evaluation, the panellists concluded that Ajwain OT 0.75 mL had the highest ranking in terms of overall pleasantness of scent and a stronger aroma of thyme. Regarding colour, the OT samples were considered superior to both the HAB samples and the control. In contrast, Ajwain OT at a dosage of 0.75 mL and Thyme OT at a dosage of 1.5 mL received the highest grade in terms of overall look. In addition, Ajwain OT 0.75 mL and 1.5 mL achieved the top scores in the comprehensive assessment of odour, hue, and flavour. For recommendations in future research, a larger number of trained sensory evaluators can support more evidence in identifying the best treatment to be used for dried jerky applied with essential oils. Furthermore, it is also highly suggested to conduct a shelf-life test to observe the antimicrobial activity of the applied essential oils on dried jerky and identify the suitable and consumer accepted dose of essential oils.

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